PROCEEDINGS

OF THE

NATIONAL ACADEMY OF SCIENCES

INDIA

1965

Vol. XXXV SECTION - A Part III

SYMPOSIUM ON LAND FERTILITY IMPROVEMENT BY BLUE GREEN ALGAE

PREFACE

Five years ago in my presidential address to the Indian Science Congress I stated that population explosion is certainly more dangerous in the long run than the atomic weapons.

In a recent article the late Adlai Stevenson recorded that bombs, babies and bulldozers are baffling human enterprize.

The food situation in this country is certainly acute, but, we must help ourselves in improving the position.

Today, in several industrially backward countries, approximately 1500 k, cals. as against the required 2800 k. cals. are being derived from national diets per capita per day. Hence, the greatest need of the world is to produce more food which can be obtained by application of nitrogenous fertilizers and manures.

At the present moment 1000 million tons of cereals and 700 million tons of other food materials and 1800 million tons of fooder are being produced in the whole world. It is well known that 1 kg. of nitrogen applied in cereal or grass production in temperate countries produces 15 to 17 million tons of cereals or grasses. In tropical countries the yield is less and is of the order of 10 kgs. per kgm. of nitrogen applied. Hence, for the production of 3500 million tons of food and fodder, approximately 250 million tons of available nitrogen are being consumed and most of it is coming from the humus of the soil as industrial nitrogen is being utilized all over the world to the extent of 10 million tons only. Legumes supply 5 million tons of nitrogen to the world soils. Hence, the fertility of the existing lands has to be considerably improved.

Keeping this in view, the National Academy of Sciences (India) Allahabad, since its inception, has concentrated on this problem and several Symposia have been held and many original investigations published in the last 30 years.

In recent years some Japanese soil scientists have claimed that blue green algae can fix nitrogen in the soil and leads to increased land fertility improvement and paddy production. But European soil scientists have clearly asserted that in temperate climates the performance of blue green algae in fertility improvement is practically nil.

Consequently, a symposium was organised by the National Academy of Sciences (India) Allahabad, on land fertility improvement by blue green algae and elgal and other organic substances and several papers were read and discussed, which are being published in this volume.

N. R. Dhar

LAND FERTILITY IMPROVEMENT BY FIXING ATMOSPHERIC NITROGEN ON APPLYING ORGANIC MATTER AND PHOSPHATES WITH AND WITHOUT ALGAE

By

N. R. DHAR

Sheila Dhar Institute of Soil Science, University of Allahabad

ABSTRACT

Yield of crops by adding nitrogenous fertilizers is much greater than that obtained by applying phosphate or potash. But the world production of industrial N today is about 10 million tons whilst the food, fodder and fibre production requires 250 million tons of N. Hence, soil humus is supplying most of the N requirement of crops in the world.

A new method of fixing atmospheric N in the soil by the slow oxidation of all types of organic matter has been discovered by us. This N fixation has been found to be accentuated by light absorption and addition of phosphates.

Excellent crop production has been obtained in normal and alkaline soils by this method all over the world.

Moreover, humus-rich or compost-rich soils can produce large crops by applying nitrogenous fertilizers.

In composting of all types of organic matter including waste coal, the addition of phosphates including phosphate rocks and Thomas slags, produce richer composts than the ordinary Howard compost. In this process also, the absorption of sunlight increases the N content of the compost.

Comparative experiments on N fixation by blue green algae show clearly that they produce a small increase of N in the soil. But, organic matter increases the N status more markedly than the blue green algae.

Experiments have been carried out by us on the loss of total N from soils mixed with either ammonium sulphate or sodium sulphate or sodium nitrate. Our observations show that in all the systems the total N decreases with lapse of time. But, in the presence of algae, there is slight checking of the N loss. With phosephates the loss is checked a little more but organic substances like wheat straw retard the loss of N markedly. These N loss experiments can explain the low recovery of nitrogenous fertilizers in crop production all the world over.

We have obtained experimental results in support of the viewpoint that humus loss in soils is accentuated by applying nitrogenous fertilizers, but, wheat straw is an excellent fixer of atmospheric N in the process of its composting or when ploughed in the soil and it is also a retarder of N loss from soils.

Experimental results show that direct ploughing in of organic matter with or without phosphates produces more crops than from the composts obtained from the same organic matter. This is due to the fact that direct ploughing in produces more N fixation in the soil than in composting where marked losses of N have been reported.

Plant leaves, all kinds of straw, grasses and specially forest litters form valuable soures of energy materials for fixing atmospheric N aided by calcium phosphates.

Hans Jenny and S. P. Raychaudhuri have reported that comparison of Indian with American soils, particularly those of California, Texas, Atlantic coast, showed an unquestionable superiority of the former over the latter when sites having equal mean annual temperatures were compared. But the Indian soils had much lower N and C contents than the tropical soils of Central and South America.

Moreover, the average N content of soils of Ootacamund and Kodaikonal, which are hill stations in the South of India near the Equator, is 0. 335% and 0.332% respectively, whilst the North Indian hill stations of Simla have 0.241% N and in Mussoorie it is 0.266% N. Similarly, Ambala and Aligarh, lying in the Northern India plains, show a N status of 0.036% N and 0.044% N, whilst Madras and Madura in the South near the Equator have 0.054% N and 0. 062% N respectively. The rainfall and temperatute in all these stations are about the same.

These observations showing greater N status of land near the Equator support the photochemical viewpoint of N fixation. Undoutedly, world soil N is created by the fixation of atmospheric N in the slow oxidation of organic matter aided by sunlight absorption and phosphates.

Due to population explosion in various parts of the world, food production has to be largely increased and hence every attempt is being made to obtain more nitrogenous substances.

NITROGEN - KEY ELEMENT IN CROP PRODUCTION

Nitrogen is considered to be the key element in crop production as is evident from the following figures showing the yield in kilograms for an application of 1 kg. of plant nutrient : –

Country.		Crops in rotation				Permanent pastures		
		N	P	K		N	P	K
Norway	...	9	3	5	...	11	6	4
Sweden	...	14	11	7	...	14	11	7
Denmark	...	18	4	2	...	12	5	3
U. K.	...	16	5	5	...	...	...	...
Ireland	...	20	8	8	...	...	...	...
Holland	..	19	6	3	...	10	6	4
France	...	19	5	2.1	...	...	—	...
Germany	...	19	8	4	...	9	10	...
Switzerland	...	18	8	4	...	9	10	5
Greece	...	15	5	3	...	...	...	...
Italy	...	11	3	.	...	12	4	3
Average :		16	8	4	...	11	7	4

In the Oxford Economic Atlas of the world (Oxford University Press, 1959), the following increased yields per kg. nitrogen are recorded :—

	Wheat	Rice	Potatoes	Grass (or hay)
Kg. per hectare :	17	17	84	17

The Indian Council of Agricultural Research has reported that the average production of paddy in India is tentimes the nitrogen amount applied. It appears that the humus content of the soil determines appreciably the response of crops to fertilizer nitrogen. The United Nations Korean Reconstruction Agency has stated that 1 kg. of nitrogen as ammonium sulphate produces 12 to 14 kg. of brown rice and 14 to 18 kg. of rough barley in South Korea. Moreover, 1 kg. of phosphate as superphsphate can yield 4 to 5 kg. of brown rice.

It has been reported that in 1958-59, 8.75 million tons of nitrogen, 9.03 million tons of phosphate and 7.83 million tons of potassium were produced by the world fertilizer industries, and the average N : P_2O_5 : K_2O ratio of fertilizers applied has shifted from 1 : 3.85 : 0.77 in 1900 to 1 : 1.03 : 0.9 in 1958-59.

From a survey of crop production and fertilizer application in different countries, it appears that in countries not using large amounts of fertilizers, the nitrogen response to cereals is very marked ; the law of diminishing returns is in operation in Holland, Belgium and Norway where large amounts of nitrogen per acre are applied. But in Japan, China and Taiwan, where composts and other plant and animal organic wastes are largely utilised with fertilizers, better crop yields per unit of nitrogen are still obtained.

INDUSTRIAL NITROGBN INADEQUATE FOR WORLD FOOD PRODUCTION

Approximately 1000 million tons of cereals, 700 million tons of pulses, potatoes, vegetables, sugars etc. are being produced in the world annually, and, the world grassland production is about 1600 million tons. Hence, assuming the yield is 15 times the nitrogen application, the production of the world's food, fodder and fibre requires approximately 250 million tons of available nitrogen per annum. It has been already stated that the industrial supply of nitrogen is only 8.75 million tons, legumes contribute 5 million tons and precipitation 7-10 million tons of nitrogen. Farmyard manure may supply 5 million tons. Hence, the world soil humus is still the chief supplier of crop nitrogen.

LARGE AMOUNTS OF NITROGEN FIXATION IN SOILS BY THE SLOW OXIDATION OF ORGANIC MATTER AIDED BY LIGHT AND CALCIUM PHOSPHATES

As nitrogenous fertilizer produced is not adequate to meet the world food situation, we have developed an alternative method of fixing atmospheric nitrogen directy in the soil by ploughing in all types of organic substances like molasses, straw, leaves, grasses, sawdust, farmyard manure, water hyacinth (Eichhornia crassipes), lucerne, KANS (Sacchrum spontaneum), cactus, municipal wastes, peat, lignite bituminous coal etc. mixed with bonemeal, rock phosphate or Thomas (basic) slag. So far organic matter and phosphates have been utilised separately, but, we have discovered that the two together are highly effective. A few of our laboratory experiments and field trials are recorded below.

A Swedish clay soil collected from the fields of the Royal College of Agriculture, Upprsala-7, containing 0.147% N, 1.207%C, 1.19% CaO, 3.114% MgO, 1.2% K_2O and 0 225% P_2O_5 (of which 0.083% is available), was utilised and the following results were obtained in dishes by mixing organic matter with soil and exposing one set to artificial light for 8 hrs. daily and another set covered with thick black cloth to exclude light. The moisture content was maintained at 20-25% by adding distilled water every alternate day :—

FIXATION OF NITROGEN IN SWEDISH SOIL

Period of exposure. (days)	Organic carbon %	Total nitrogen. %	Carbon oxidised. %	Increase in nitrogen. %	Efficiency : nitrogen fixed in mg. per gram of C oxidised
LIGHT		Swedish soil + sucrose.			
0	2.3568	0.1470	...	...	...
150	1.4833	0.1656	0.8735	0.0186	21.3
300	1.2723	0.1682	1.0845	0.0212	28.4
DARK					
0	2.3568	0.1470	...	...	...
150	1.7274	0.1554	0.6321	0.0084	13.3
300	1.5036	0.1598	0.8532	0.0128	15.0
	Swedish soil + 0·25% P_2O_5 as Gafsa rock phosphate + sucrose				
LIGHT					
0	2·3568	0·1470	...	...	...
150	1·4132	0·1862	0·9436	0·392	41·5
300	1·1518	0·1992	1·2050	0·0522	43·4
DARK					
0	2·3568	0·1470	...	...	...
150	1·6787	0·1608	0·6781	0·0138	20·3
300	1·4612	0·1658	0·8956	0·0188	21·0

Fixation of nitrogen in Allahabad soils.

Period of exposure. (days)	Organic carbon %	Total nitrogen. %	Carbon oxidised. %	Nitrogen fixed. lb/acre.	Efficiency.
		Allahabad soil + wheat straw.			
LIGHT					
0	0·7356	0·0492	...	...	...
90	0·5358	0·0533	0·1998	...	20·8
150	0·4762	0·0544	0·2594	...	20·6
180	0·4365	0·0553	0·2991	117·6	20·6
DARK					
0	0·7356	0·0492	...	...	...
90	0·5866	0·0507	0·1490	...	10·6
150	0·5417	0·0511	0·1939	43·7	10·1
180	0·5241	0·0513	0·2115	...	10·2
	Allahabad soil + Wheat straw + 0·1% P_2O_5 as $Ca_3(PO_4)_2$.				
LIGHT					
0	0·7356	0·0492	...	...	...
90	0·4924	0·0566	0·2432	...	30·9
150	0·4181	0·0588	0·3175	215·2	30·3
180	0·3740	0·0602	0·3616	...	30·6
DARK					
0	0·7356	0·0492	...	...	...
90	0·5513	0·0522	0·1843	...	16·3
150	0·4851	0·0532	0·2505	90·0	16·0
180	0·4652	0·0534	0·2704	...	15·8

MECHANISM OF NITROGEN FIXATION

These organic substances undergo slow oxidation in air when mixed with soil even in the dark as the amount of carbonaceous compounds decreases with lapse of time. In these slow oxidations energy is liberated which is utilised in fixing atmospheric nitrogen on the soil surface forming ammonia, amino acids and

other nitrogenous compounds. The energy liberated in the oxidation of glucose ($C_6H_{12}O_6+6O_2=6CO_2+6H_2O+676$ k, cal.) breaks up a gram mole water into H and OH (H_2O+112 k. cal.$=H+OH$). The atomic hydrogen produced combines with nitrogen and forms NH_3 which can be readily oxidised to nitrite and nitrate by air or H_2O_2 obtained from OH radicals. The nitrate readily reacts with organic matter and forms amino acids. When these systems are illuminated by sunlight or artificial light, the light is absorbed, and the nitrogen fixed in presence of light is much greater than that obtained in the dark. In all these experiments the number of Azotobacter, total bacteria and fungi are always smaller in presence of light· which is harmful to microorganisms, than in the dark, although the nitrogen per gram of carbon of the energy material oxidised in light is much greater than in the dark. In presence of calcium phosphates, the nitrogen is greatly enhanced.

In our experiments with molasses, sucrose and other soluble carbohydrates, there is marked increase of ammoniacal nitrogen as well as of total nitrogen in a short time; but with cellulosic materials like straw, liberation of ammonia from the system takes place in a longer time. Consequently, a time. interval of 2 to 6 months according to the soil temperature is needed between the incorporation of straw or materials rich in cellulose and lignin in the soil and the sowing of a crop depending chiefly on the soil temperature and humidity. In this process Thomas (basic) slag, being alkaline and containing chemical catalysts, helps in the partial oxidation of the organic substances and the liberation of ammonia and formation of nitrate.

Analysis of different soil samples from the same field.

Treatment	Organic carbon %	Total nitrogen %	C/N ratio
No slag	... 1·90	0·172	11·0
Tata basic slag	... 1·77	0·331	5·3
Tata basic slag	... 2·01	0·270	7·4
Tata basic slag	... 1·89	0·279	6·7

Allahabad municipal waste was dumped on a field with or without Tata basic slag containing 8% P_2O_5 with the above results and bumper crops were obtained in this improved land.

Karraker, working in the U. S. A., obtained the following results showing marked nitrogen fixation and improved crop yield by a mixture of manure and phosphate:—

Average of 3 field treatments		Nitrogen in soil per acre (lb/acre)	Corn yield. bushels/acre
No manure	...	... 1600	17
Manure	...	... 1760	36
Manure + Phosphate	...	... 1990	51

From a survey of the phosphate status of world soils it appears that alkaline phosphates like Thomas slags, rock phosphates and even animal dung containing phosphate, show more residual effect than the soluble varieties of phosphates like superphosphate, chiefly because calcium carbonate present in the alkaline phosphate and farmyard manure markedly check the washing away of phosphates from soils. Some of our field trials in Bengal with Paddy grains (kg/acre) are recorded below:—

Field experiments in Paddy and Wheat production by organic matter and phosphates

Treatments	1958-59	1959-60	1960-61	Average
1. Control ...	322·40	531·60	816·40	556·80
2. Organic matter (straw 10 tons/acre) ...	362·40	762·40	985·20	703·20
3. Basic slag (40 lb. P_2O_5/acre) ...	350·80	745·20	861·60	652·40
4. Superphosphate ,, ,, ,, ...	325·60	632·80	822·00	610·00
5. Organic matter (straw) + basic slag ...	390·00	784·00	974·80	714·80
6. ,, ,, ,, + Superphosphate ...	399·20	771·60	918·00	696·00

In the following experiments wheat was grown after harvesting paddy on the same land:—

Yield of Paddy grain in Anapur-Allahabad in kg. per 1/60 acre

Treatments	BLOCKS 1	2	3	4	5	6	Total.
Soil alone (control) ...	11·50	12·25	10·75	11·00	13·00	10·25	68·75
Soil + Wheat straw (10 tons/acre) ...	18·00	17·25	19·50	16·50	18·50	18·25	108·00
Soil + Wheat straw + P_2O_5 as Tata slag	23·25	22·75	23·50	24·50	21·50	23·50	139·00
Soil + KANS (Sacchram spontaneum) (10 tons/acre) ...	16·75	15·50	16·25	17·75	15·00	16·00	97·25
Soil + KHANS + P_2O_5 as Tata slag ...	21·75	21·25	22·25	22·75	20·50	21·25	129·75
Soil + sunhemp (Crotalaria juncea) ...	15·50	14·75	16·75	15·25	15·75	16·00	94·00
Soil + sunhemp + P_2O_5 as Tata slag ...	18·75	19·25	17·50	18·50	19·50	18·50	112·00
Total ...	125·50	123·00	126·50	126·25	123·75	123·75	748·75

Yield of Wheat grain (kg.)

Soil alone (control) ...	8·25	10·50	9·25	8·00	10·50	10·00	56·50
Soil + Wheat straw (10 tons/acre) ...	13·25	13·25	13·50	13·00	14·50	14·50	82·00
Soil + Wheat straw + P_2O_5 as Tata slag (50 lb/acre) ..	19·25	18·50	20·00	17·50	19·00	17·25	111·50
Soil + KANS (10 tons/acre) ...	12·50	13·00	11·50	14·00	12·25	13·25	76·50
Soil + KANS + P_2O_5 as Tata slag (50 lb/acre) ...	16·25	17·50	15·50	17·25	18·00	16·00	100·50
Soil + Sunhemp (10 tons/acre)	11·50	11·50	12·75	11·50	10·25	11·50	69·00
Soil + Sunhemp + P_2O_5 as Tata slag (50 lb/acre) ...	15·75	14·25	15·75	16·00	15·25	15·50	92·50
Total ...	96·75	98·50	98·25	97·25	99·75	98·00	588·50

Experiments in Saline & Alkaline soils of Rajasthan (India)

Organic matter (straw or pressmud) 10 tons/acre and P_2O_5 10 lbs/acre

	YIELD : (kg/acre)	
	Paddy grains	Barley grains
Control	... 45·93	93·75
Organic matter (straw)	... 126·12	175·60
„ „ „ + P_2O_5 as bonemeal	... 203·91	241·90
Pressmud	... 370·18	266·25
„ + P_2O_5 as bonemeal	... 501·62	382·50

At my request Lady Eve Balfour of The Soil Association, New Bells Farm, Haughley, Suffolk, England, performed the following experiment:—

A 5·42 acres plot of land with barley was harvested by a combine harvester on 9/9/57 and barley straw remained on the land which was ploughed up early in January 1958. To one portion of the land (3·12 acres), ammonium sulphate at 1 cwt. nitrogen per acre was applied on 17/10/57. To the second portion, nothing was added. In the third section (1·75 acres), 22% Thomas (basic) slag was applied to the straw on 16/12/57 at 99 lbs. P_2O_5 per acre. On 3/4/58, 1 ton and 4 cwt. of a compound fertilizer (Fison 35), 25 lb. nitrogen, 62 lb. P_2O_5 and 62 lb. K per acre was applied to the whole 5·42 acres. Barley was sown on 10/4/58 and harvested on 6/9/58. Lady Balfour reported that the plot to which slag was added to the straw contained the largest amount of total N and produced the biggest crop of barley. The yield of barley in the 3·12 acres plot (to which ammonium sulphate was applied) was 20·4/5 cwt. per acre. The control plot (to which nothing was added) gave 14 cwt. per acre. But in the third plot containing Thomas (basic) slag, the yield of barley was the highest, i. e. 30·2/7 cwt. per acre.

The Inter-African Bureau of Soils are also utilising straw and basic slag in Africa on a large scale.

It is good for world agriculture that G. Bjalfve of the Royal College of Agriculture, Sweden, has also observed (private communication) marked N fixation more in light than in the dark by incorporating straw with soil or sand and that calcium phosphates largely increase this N fixation. Bjalfve believes that this photochemical and thermal fixation of N may be of greater importance all over the world than legumes which are difficult to grow. Crop production in many countries without manures or fertilizers and the marked fertility of prairie lands can be satisfactorily explained from the viewpoint of N fixaticn observed by us from the oxidation of plant residues and grasses incorporated in the soil aided by light and phosphates.

As large amounts of plant materials are photosynthesised on the earth's surface, there is no dearth of organic matter. Moreover, the estimated world reserves of rock phosphate deposits are of the order of 21000 million tons and the world consumption is only 700 million tons per year. The world steel industry is producing increasing amounts of phosphate as basic slag. Hence, large amounts of nitrogen fixation and land fertility improvement on a worldwide scale are possible by adopting this method by utilising cereal straw, grasses, forest litters, leaves etc. as energy materials.

In France the use of Thomas (basic) slag which is cheap is increasing and is taking the place of superphosphate. It has been reported that basic slag appreciably increases available N in the system and doubles grass yields.

Sir John Russell, in WORLD POPULATION & WORLD FOOD SUPPLIES, 1954, p. 7, has recorded as follows: "...And in all countries, even in the most advanced, there is an equally great or even larger gap between the present achievements of the best farmers and the high yields occasionally obtained as the result of the combination of factors which in nature occur only rarely but which, if we could discover and reproduce them, would make these same records a more usual, perhaps even the normal occurrence."

Prof. Bradfield, in his presidential address (1960 VIIth International Soil Science Congress, Madison, Opening Session, 5, XXIX), has also recorded high yields of cereals in different parts of the world. A land in Iowa kept under blue grass for 50 years, when ploughed up and phosphated, produced 224 bushels of corn per acre. It appears therefore that tne growing of blue grass creates prairie lands with 0·6% total N and over 500 lbs. of available N per acre. In experiments in India large crop yields of cereals and potatoes have been obtained by adding large amounts of composts aided by nitrogenous fertilizers. The scientific experimental observations in different parts of the world show that humus-rich soils can produce bumper crops. In the 'Fen' soils containing 3% total nitrogen and in the 'muck' soils in New Jersey containing 2·7% N, large crops are produced aided by fertilizers.

PHOSPHATED COMPOSTS

In composting waste organic materials we have observed a very marked effect of phosphate and light in improving the value of composts in plant growth. This is evident from the following results obtained in composting dry leaves with and without phosphate in big wooden boxes: —

Dry leaves of Guava (Psidium guajava)

Days	Total carbon (gm.)	Total N_2 (gm.)	NH_4-N_2 (gm.)	Available NO_3-N_2 (gm.)	Total avail N over total N %	C/N ratio	Available P_2O_5 (gm.)	pH.	Weight of compost (kgm.)
0	12163·00	262·36	...	...	...	46·30	...	...	35·00
50	9730·44	311·02	10·136	11 946	7·1	31·28	22·568	6·9	30·68
100	7361·08	348·45	15·826	19·716	10·2	21·12	31·013	6·0	27·11
150	6281·15	374·62	20·443	30·505	13·6	16·76	38·646	5·4	24·28
200	5401·66	365·14	15·707	24·267	10·4	14·79	42·291	6·3	22·04
Dry leaves + 1% P_2O_5 as Tata basic slag.									
0	12163·00	262·36	...	...	...	46·30	189·30	...	39·88
50	9536·64	317·84	14·636	17·668	10·1	30·00	238·40	7·8	33·12
100	6655·50	404·51	25·801	34·066	14·8	16·45	269·50	6·4	29·08
150	5415·04	484·62	34·098	48·772	17·1	11·17	281·10	5·8	26·76
200	4780·00	468·62	28·925	41·368	15·0	10·20	290·80	6·9	30·18
Dry leaves + 20% coal.									
0	12163·00	262·36	...	...	...	46·30	...	...	42·00
50	13681·25	415·29	8·807	10·075	7·54	32·90	20·708	7·2	38·08
100	11542·63	456·16	13·542	16·874	6·67	25·30	28·553	6·5	34·92
150	9911·55	478·65	17·293	23·778	8·58	20·70	36·118	6·0	32·11
200	8588·08	468·70	14·156	19·278	7·14	16·30	40·065	6·6	30·18
Dry leaves + 20% coal + 1% P_2O_5 as Tata basic slag									
0	16045·75	375·08	...	..	...	42·90	189·30	...	46·88
50	13271·17	437·31	11·777	15·369	6·21	30·30	230·10	8·0	42·13
100	11007·20	479·42	20·648	29·406	10·44	22·90	248·70	7·0	39·34
150	9144·61	501·41	28·912	40·051	13·76	18·30	260·20	6·6	36·01
200	8010·40	493·33	25·154	35·002	12·22	16·20	268·60	7·0	33·27

Yield of Paddy and Wheat grains in pots from Composts obtained from dry leaves mixed with coal and Tata basic slag

Treatments		Total yield in grams. Paddy grain.	Wheat grain
1. Soil alone (control)	... ...	89	48
2. Soil + Unphosphated compost	... ...	188	102
3. „ + „ „ containing 20% coal	... ...	208	117
4 Soil + Phosphated compost	... ...	257	132
5. „ + „ „ „ containing 20% coal	...	308	143

The above results clearly show that the addition of bituminous waste coal improves the plant-producing power of a compost.

Moreover, in composting Allahabad municipal waste, the following results were obtained:—

Composition of Allahabad municipal waste compost.

	Phosphated municipal compost	Unphosphated municipal compost
Total P_2O_5	... 1·2156	0·8525
Available P_2O_5	... 0·3650	0·2453
K_2O ...	... 0·8054	0·7521
MgO ...	... 0·2615	0·2506
CaO ...	... 3·2162	1·8425
Total carbon	... 4·1600	6·2700
Total nitrogen	... 1·2500	0·8242

Yield of Barley grains in pots from the above Compost.

Treatments		Total yield (gm.)
1. Soil alone (control)	... ...	50
2. Phosphated municipal waste	(5 tons/acre) ...	181
3. „ „ „	(10 tons/acre) ...	253
4. Unphosphated „ „	(5 tons/acre) ...	133
5. „ „ „	(10 tons/acre) ...	193

From times immemorial organic matter has been profitably applied in crop production. Since 1880 Thomas (basic) slag is being used as a phosphatic fertilizer. But, Dhar and coworkers have discovered that for crop production hoth, organic matter and Thomas slag, should be ploughed in mixed because the two together are ever so much better than either of them alone. Wheat straw or other cereal straw when ploughed in can fix atmospheric N and supply available N, potash and phosphate for crop growth, but, when mixed with Thomas slag, the N fixation is greatly increased and more available N, potash, phosphate and trace elements are supplied to crops. Thomas slag, being alkaline due to its contents of CaO, K_2O, MgO etc., oxidation of cellulose and lignin materials in straw is greatly increased when it is mixed with straw and ploughed in the soil. Moreover, chemical oxidation catalysts like Cu, Fe, Mn, Ti, Mo, V, Zn in the slag also accelerate oxidation of cellulose. In this process energy is liberated and utilised in fixing atmospheric N on soil surface and land fertility is markedly increased. Oxidation of carbonaceous compounds of soil humus and those added by ploughing in organic matter like straw is greatly facilitated by adding Thomas slag and this is a very important function of the slag. In this way not only the total N of the system increases by ploughing in Thomas slag and straw but, due to increased oxidation in the soil by adding Thomas slag, the available N, phosphate, potash, lime, magnesia etc., that is, all important plant nutrients increase considerably making the land more productive. This happens not only with the phosphorus poor soils but in all soils, and this method is applicable all over the world. Trace elements present in the slag form an important part in plant nutrition. In temperate countries the interval between the ploughing in of straw and Thomas slag and in the sowing of the next crop should be 2 to 3 months. By this time the carbonic acid and the organic acids produced in cellulose oxidation make more potash, phosphate, lime, magnesia available to crops.

Dhar, in his presidential address to the National Academy of Sciences, India, on 15/1/37, first emphasised that direct ploughing in of organic matter is certainly better than the addition of the compost obtained from organic matter because in the direct ploughing in of the organic matter there is much more N fixation than in composting where even marked losses of N have been reported by soil scientists. In recent years a very large number of comparative experiments have been carried on by the direct ploughing in of various organic substances with and without phosphates and the compost obtained from the organic substances either phosphated or not phosphated. In the following table the comparative results obtained by us have been recorded:—

Yield of Paddy grain (kgms.)

	Treatments		Organic materials added directly Mean of 4 replications	Organic materials added after composting Mean of 4 replications
1.	Soil alone	...	46·3	46·1
2.	Soil + Wheat straw	...	74·3	66·3
3.	Soil + „ „ + Tata Thomas slag	...	96·6	88·7
4.	Soil + Paddy straw	...	69·6	61·3
5.	Soil + „ „ + Tatata Thomas slag	...	89·3	81·8
6.	Soil + KANS (Sacchram spontaneum)	...	65·3	57·6
7.	Soil + KANS + Tata Thomas slag	...	84·6	76·8
8.	Soil + mixed leaves	...	62·1	5[illegible]·3
9.	Soil + „ „ + Tata Thomas slag	...	81·5	73·5
10.	Soil + Jack fruit leaves	...	60·2	52·4
11.	Soil + Jack fruit leaves + Tata Thomas slag	...	79·6	71·5
12.	Soil Sunhemp „ „	...	58·6	51·0
13.	Soil + Sunhemp + Tata Thomas slag	...	77·3	69·4
14.	Soil + Cactus	...	56·7	49·2
15.	Soil + Cactus + Tata Thomas slag	...	75·8	67·6

From the foregoing results it is clear that direct ploughing in of organic matter produces greater yield than from the compost formed from the same organic matter.

Lady Eve Balfour of The Soil Association, Haughley, Suffolk, England has recorded as follows:—

"Dr. Dhar's work on behalf of soil fertility is known in many countries. Particularly valuable to world agriculture is his discovery that crude organic. matter such as straw left by combine harvesters can be safely ploughed in with it Dr. Dhar is deeply concerned at the declining humus status of so much of the agricultural soils of the world, which is at its worst of course in the big grain producing areas and it is there that Dr. Dhar's discovery is of such special importance in that it provides a practical alternative to burning of straw. Here at

Haughley we use Dr. Dhar's method when ploughing in straw on our stockless section. We have done so regularly since our first experiment with it in 1957-58."

Lady L. E. Howard has stated: "In India's sunny climate fixation of atmospheric N is a most advantageous factor. Prof. Dhar's researches on this point have opened up new possibilities of the greatest importance. Fixation is greatly increased if the material is treated with phosphates, preferably in the inorganic form as phosphate rock or Thomas slag; if so phosphated, percentage of N in municipal wastes when composted rises from 0·64% to 1·02% and available phosphate from 0·13% to 0·18%. On world basis Prof. Dhar has also estimated that as much as 8 to 10 million tons of N, 4 to 5 million tons of phosphate and about the same amout of potash could be recovered from municipal wastes."

Dr. C. T. Jones, Interprovincial Patents Limited, Ste-314-718, Granville St., Vancouver 2, B. C., Canada, writes: "We are quite interested in your work and firmly believe that its application in this country would be of great benefit...The soils are mostly alkaline and large areas are subject to erosion brought about by over-cropping and lack of humus. It has been the practice to burn off the straw residues after combining, so little organic matter has been returned to the soil...It can be readily seen that while any synthetic fertilizers are used under these conditions, it is only a matter of time before these lands become dust bowls, which in fact, some already are...We would welcome a suggestion from you whereby your background of experience in this field may be used to further enhance the value of our products on mutually satisfactory basis."

Dr. Richard K. Houston, Kansas City, Missouri 64112, U. S. A., writes:—

"I have read with great interest your researches on chemical fixation of nitrogen. I am interested in the application of these principles to agricuture in the U. S. A., particularly lawns...Some of the Coal Companies I am in contact with are interested in using coal agriculturally. It is quite possible that in researching the use of coal for a turf market in the U. S. A. we may well be of service to one another...I feel your work to be very important because if we can increase the N conversion of crop residues from 10 lbs. of N per 1000 lbs. of C to 60 lbs. of N, this would allow the small farmer with no capital to produce his own nitrogen. I believe the U. S. Govt. would be very interested in helping finance research on this approach with counterpart funds."

NITROGEN FIXATION WITH ALGAE AND ORGANIC MATTER

It has been believed from a long time that some blue green algae increase land fertility and reclaim alkali land chiefly by their power of fixing atmospheric N. Dr. Watanable of Japan and Dr. P. K. De and Dr. R. N. Singh of India are the chief exponents of this viewpoint. On the other hand Russell has

stated unequivocally that in temperate climates there is no evidence of fertility improvement by the blue green algae. So, in order to throw further light on this subject comparative experiments have been carried on by inoculating soils with (a) Anabena naviculoides, (b) Tolypothrix tenuis and (c) Chlorella vulgaris.

250 gms. of black cotton soil collected from the Agricultural College Farm, Indore (Central India) were mixed with algae, with and without basic slag. Sunhemp and wheat straw were utilised as energy materials and 40% moisture was maintained in all the 'sets' throughout the experimental period. The results obtained are given below:—

Analysis of soil, organic materials and basic slage used in the experiment.

Estimation		Soil (oven-dry basis %	Wheat straw %	Sunnhemp %	Tata basic slag %	German basic slag %
Loss on ignition	...	...	89·9040	92·9200	...	...
Ash ...	...	...	9·0940	7·0800	...	...
HCl insoluble silica	...	57·6600	5·0450	2·9460	15·6846	11·4665
Sesquioxide	...	28·9451	1·4168	0·7614	...	...
Fe_2O_3 ...	...	18·6740	0·6172	0·3553	15·5650	14·8760
Al_2O_3 ...	...	10·3801	0·7996	0·4061	5·4320	3·0678
Total CaO	...	2·8464	0·8432	1·1080	38·6946	42·3467
Total MgO	...	1·0567	0·4081	0·3206	4·8486	4·9684
Total K_2O	...	0·8313	0·8014	0·7824	0·6474	Traces
Total P_2O_5	...	0·1452	0·6036	0·7245	7·1600	17·2600
Available P_2O_5	...	0·0045	...	...	4·0520	9·9040
Total carbon	...	0·4800	38·2650	29·8700	...	...
Total nitrogen	...	0·0458	0·6370	2·2200	...	...
Ammoniacal nitrogen	...	0·0014	0·0	...	...	...
Nitrate nitrogen	...	0·0018	...	...	...	...
C/N ratio	...	10·62	60·07	17·96	...	...
$CaCO_3$	...	3·7500	...	...	...	...

Experiment with Black soil.

Treatments	60 days	120 days	180 days	240 days
Soil alone	0·9	1·6	1·8	1·5
Soil+Chlorella	1·0	1·4	1·8	1·6
Soil+Anabaena	1·4	2·0	2·3	1·9
Soil+Tolypothrix	1·5	2·1	2·5	1·1
Soil+Tata basic slag	1·1	1·8	2·1	2·6
Soil+ ,, ,, ,, +Chlorella	1·2	1·8	2·2	1·8
Soil+ ,, ,, ,, +Anabaena	1·6	2·3	2·8	2·1
Soil+ ,, ,, ,, +Tolypothrix	1·7	2·4	3·0	2·4
Soil+German basic slag	1·2	1·9	2·2	1·9
Soil+ ,, ,, ,, +Chlorella	1·3	1·9	2·4	1·9
Soil + ,, ,, ,, +Anabaena	1·8	2·5	3·2	2·7
Soil+ ,, ,, ,, +Tolypothrix	1·9	2·8	3·4	3·0
Soil+Sunnhemp	7·7	11·2	13·7	10·6
Soil+ ,, ,, +Chlorella	7·9	11·5	13·9	11·0
Soil+ ,, ,, +Anabaena	9·9	14·1	19·4	15·3
Soil+ ,, ,, +Tolypothrix	10·3	14·5	20·2	17·3
Soil + ,, ,, +Tata basic slag	16·0	21·3	25·3	22·8

Soil + ,, ,,	+ ,, ,, ,, +Anabaena ...	18·2	26·9	31·6	29·1
Soil+ ,, ,,	+ ,, ,, ,, +Chlorella ...	16·2	21·6	25·6	23·4
Soil+ ,, ,,	+ ,, ,, ,, +Tolypothrix ...	18·6	27·7	32·4	30·3
Soil+ ,, ,,	+German ,, ,, ...	16·8	22·8	25·8	23·4
Soil+ ,, ,,	+ ,, ,, ,, +Chlorella ...	17·1	23·0	26·2	23·9
Soil+ ,, ,,	+ ,, ,, ,, +Anabaena ...	19·3	28·2	32·4	30·1
Soil+ ,, ,,	+ ,, ,, ,, + Tolypothrix ...	19·8	29·3	34·0	31·5
Soil+Wheat straw	... ...	7·9	11 8	14·1	12·3
Soil+ ,, ,,	+Chlorella	8·2	12·0	14·4	12·5
Soil+ ,, ,,	+Aabaena	10·5	16·0	20·2	17·7
Soil+ ,, ,,	+Tolypothrix	11·1	16·6	20·9	18·6
Soil + ,, ,,	+Tata basic slag. ...	18·2	23·6	26·6	24·4
Soil+ ,, ,,	+T.B.S.+Chlorella ...	18·7	23·8	27·0	25·0
Soil+ ,, ,,	+ ,, +Anabaena ...	19·4	28·6	33·2	31·3
Soil+ ,, ,,	+ ,, +Tolypothrix ...	19·9	28·9	34·3	32·7
Soil + ,, ,,	+German basic slag ...	19·1	24·8	27·2	25·1
Soil+ ,, ,,	+G.B.S.+Chlorella ...	19·4	25·0	27·6	25·6
Soil+ ,, ,,	+ ,, +Anabaena ...	20·5	30·0	34·2	32·7
Soil+ ,, ,,	+ ,, +Tolypothrix ...	20·9	:0·9	35·5	34·2

The above table shows increases in total N of the system at different time intervals. These conclusively prove that the increase in N status of the soil is much greater with organic matter, specially in presence of phosphorus than with algae, which produce a very small increase of N. The above results show that after 240 days there is appreciable loss of N fixed. Moreover, experiments were also carried out to study the appreciable loss of N from systems containing the same soils treated with 0.1% N in the form of ammonium sulphate or sodium nitrate.

Loss of N from soils on applying fertilizeres and its retardation by Organic matter.

N loss (%) with ammonium sulphate.

Traatments			60 days	120 days	180 days	240 days
Soil alone	...	...	50.1	62.2	70.4	74.0
Soil+Chlorella	...	...	47.3	57.0	64 2	70.2
Soil+Anabaena	...	...	49.3	59.3	66.7	72.1
Soil+Tolypothrix	...	...	48.7	58.5	65.8	71.4
Soil+T. B. S.	...	...	47.8	59.0	65.8	71.7
Soil+ ,, +Chlorella	...	...	44.6	55.8	62.3	66.2
Soil+ ,, +Anabaena	...	...	46.1	57.5	64.6	68.6
Soil+ ,, +Tolypothrix	...	...	45.8	56.4	63.7	67.4
Soil+G. B. S.	...	...	45.6	56.1	63.2	69.1
Soil+ ,, +Chlorella	...	...	42,1	51.9	58.5	63.1
Soil+ ,, +Anabaena	...	...	44.0	55.7	61.5	65.4
Soil+ ,, +Tolypothrix	...	...	43.3	53.7	60.1	64.2
Soil+Sunnhemp	...	...	40.7	50.3	55.7	60.5
Soil+ ,, +Chlorella	...	...	36.9	46.7	50.5	54.9
Soil+ ,, +Anabaena	...	...	38.8	49.3	53.0	57.7

Soil+ ,, +Tolypothrix	...	...	37.6	48.2	51.7	55.5
Soil+ ,, +T. B. S.	...	...	36.6	46.1	51.2	55.4
Soil+ ,, + ,, +Chlorella	...	...	33.4	42.0	47.7	50.1
Soil+ ,, + ,, +Anabaena	...	...	34.8	43.4	48.2	52.6
Soil+ ,, + ,, +Tolypothrix	...	...	34.3	42.9	48.0	51.6
Soil+ ,, +G. B. S.	...	...	34.1	43.7	48.9	53.4
Soil+ ,, + ,, +Chlorella	...	...	31.9	38.4	44.8	48.0
Soil+ ,, + ,, +Anabaena	...	...	32.6	40.0	46.8	50.3
Soil+ ,, + ,, +Tolypothrix	...	...	32.3	39.2	45.7	49.0
Soil+Wheat straw	...	...	38.9	48.6	54.1	58.4
Soil+ ,, ,, +Chlorella	...	...	32.9	43.5	48.8	52.8
Soil+ ,, ,, +Anabaena	...	...	37.4	47.0	51.3	55.8
Soil+ ,, ,, +Tolypothrix	...	...	35.4	45.8	50.5	54.9
Soil+ ,, ,, +T. B. S.	...	...	35.8	45.2	50.4	53.9
Soil+ ,, ,, + ,, +Chlorella	...	...	32.8	39.3	44.5	47.8
Soil+ ,, ,, + ,, +Anabaena	...	...	34.5	41.0	47.6	50.4
Soil+ ,, ,, + ,, +Tolypothrix	...	...	33.4	40.6	46.2	49.0
Soil+ ,, ,, +G. B. S,	...	...	33.6	43.0	48.0	52.5
Soil+ ,, ,, + ,, +Chlorella	...	...	31.4	38.2	43.7	47.0
Soil+ ,, ,, + ,, +Anabaena	...	...	32.1	40.8	46.1	49.7
Soil+ ,, ,, + ,, +Tolypothrix	...	...	31.7	39.2	45.5	48.3
Soil+ ,,						

Nitrogen loss (%) with Sodium nitrate

Treatments	60 days	120 days	180 days	240 days
Soil alone	22.8	26.0	28.5	30.3
Soil+Chlorella	18.3	23.0	25.7	27.2
Soil+Anabaena	19.2	23.9	26.6	28.4
Soil+Tolypothrix	18.8	23.5	26.2	28.0
Soil+Tata hasic slag	20.4	24.2	27.0	28.5
Soil+T. B. S.+Chlorella	18.0	21.5	23.4	25.0
Soil+ ,, +Anabaena	18.7	22.6	24.2	25.9
Soil+ ,, +Tolypothrix	18.5	22.0	23.8	25.5
Soil+German basic slag	19.0	22.4	25.1	27.1
Soil+G. B. S.+Chlorella	16.7	20.4	22.0	23.5
Soil+ ,, +Anabaena	17.3	21.0	22.6	24.3
Soil+ ,, +Tolypothrix	17.0	20.6	22.4	23.8
Soil+Sunnhemp	14.2	17.5	20.0	22.3
Soil+ ,, +Chlorella	12.5	15.0	17.2	19.0
Soil+ ,, +Anabaena	12.9	15.9	18.3	20.1
Soil+ ,, +Tolypothrix	12.8	15.6	17.6	19.6
Soil+ ,, +G. B. S.	10.9	14.6	16.1	18.5

Soil+ ,, + ,, +Chlorella	... ...	9.4	11.1	13.1	15.3
Soil+Sunnhemp+G.B.S. +Anabaena	... ...	10.0	11.6	13.8	16.0
Soil+ ,, + ,, +Tolypothrix	... ...	9.8	11.3	13.5	15.5
Soil+ ,, +T. B. S.	... ...	12.0	15.4	17.2	19.3
Soil+ ,, + ,, +Chlorella	... ...	10.6	12.7	14.2	16.3
Soil+ ,, + ,, +Anabaena	... ...	11.0	13.5	15 3	17.1
Soil+ ,, + ,, +Tolypothrix	... ...	10.9	13.3	14.8	16.7
Soil+ Wheat straw	... ...	12.7	15.7	17.9	20.0
Soil+ ,, ,, +Chlorella	... ...	10.8	12.6	14.3	16.1
Soil+ ,, ,, +Anabaena	... ...	11.6	13.3	15.0	17.5
Soil+ ,, ,, +Tolypothrix	... ...	11.3	12.9	14.8	16.9
Soil+ ,, ,, +T. B. S.	... ...	11.1	14.6	16.5	18.1
Soil+ ,, ,, + ,, +Chlorella	... ...	9.6	11.2	13.3	14.6
Soil+ ,, ,, + ,, +Anabaena	... ...	10.2	11.7	14.1	15.5
Soil+ ,, ,, + ,, +Tolypothrix	... ...	10.0	11 5	13.8	15.1
Soil+ ,, ,, +G. B. S.	... ...	10.0	13.3	15.1	17.7
Soil+ ,, ,, + ,, +Chlorella	... ...	8.9	10.8	12.2	13.3
Soil+ ,, ,, + ,, +Anabaena	... ...	9.3	11.5	12.9	14.2
Soil+ ,, ,, + ,, +Tolypothrix	... ...	9.0	11.1	12.6	13.9

The above results clearly show that in all these systems the total N decreases with lapse of time, but, in presence of algae the loss is very slightly checked. With phosphates the loss is checked a little more, but, with the organic substances the checking of the loss is more marked. These N loss experiments can explain the low recovery of nitrogenous fertilizers in crop production.

Thus, it is clear from these experiments that both N fixation and N loss in soils are affected more markedly by organic matter than by growing algae.

We carried on field experiments at Ramnagar, Allahabad (Innia), in the farmers' fields to study the influence of anabaena naviculoides in the growth of paddy under waterlogged conditions but no beneficial results were observed under these experimental conditions.

By taking a fairly rich Allahabad soil containing 0.214% N and 3.145% carbon and keeping it in contact with the air at 30°C, we have recently observed that in 200 days 9.5% carbon in the soil is oxidised in light, whilst in the dark the oxidation of carbon is 7.4%. When mixed with wheat straw and making the carbon content of the system 5.9%, the oxidation of the total organic C in light in the same time is 6.8% and 5.2% in the dark. When various nitrogenous substances, e. g. mustard oil cake hippuric acid, gelatine, glycine, uric acid, creatine, urea, ammonium suplphate, ammonium phosphate, ammonium persulphate and ammonium nitarate were added to the extent of 0.2% additional N, it was observed that after 200 days of oxidation in light, the carbon of the system was oxidised from 12 to 46.8%, whilst in the dark the oxidation varied from 7.9 to 43.6%. Hence, we have obtained experimental results in support of the viewpoint that humus loss in soil is accentuated by nitrogenous fertilizers. But, wheat straw has been observed to be an excellent fixer of atmospheric N in the process of its composting or when ploughed in the soil. Wheat straw is also a retarder of N loss from soils.

Plant leaves, all kinds of straw, grasses and specially forest litters form valuable sources of energy materials for fixing atmospheric N aided by different phosphates.

Hans Jenny and S. P. Raychaudhuri have reported that comparison of Indian with American soils, particularly those of California, Taxas, Atlantic coast, showed an unquestionable superiority of the former over the latter when sites having equal mean annual temperatures were compared. But the Indian soils had much lower N and C contents than the tropical soils of Central and South America.

Moreover, the average N content of soils of Ootacamund and Kodaikonal, which are hill stations in the South of India near the equator. is 0.335% and 0.332% respectively, whilst the North Indian hill stations of Simla have 0.241% N and in Mussorie it is 0.266% N. Similarly, Ambala and Aligarh, lying in the Northern India plains, show a nitrogen status of 0.036% N and 0.044% N whilst Madras and Madura in the south near the Equator, have 0.054% N and 0.062% N respectively. The rainfall and temperature in all these stations are about the same.

These observations showing greater nitrogen status of land near the Equator support the photochemical viewpoint of nitrogen fixation. Undoubtedly, world soil N is created by the fixation of atmospheric N in the slow oxidation of organic matter aided by sunlight absorption and phosphates.

INFLUENCE OF ALGAE ON NITROGEN FIXATION IN BLACK COTTON SOILS

By

N. R. DHAR *and* P. G. DEO

Sheila Dhar Institute of Soil Science, University of Allahabad

ABSTRACT

Experimental results obtained with black cotton soils of Indore show that although the blue green algae, Anabaena naviculoides and Tolyphothrix tenuis can fix some atmospheric nitrogen in the soil, the amount is small and almost negligible in comparison to that fixed by organic materials undergoing slow oxidation in presence of light and phosphates. The major source of soil nitrogen is created by the slow oxidation of organic materials obtained in plant photosynthesis aided by phosphates and sulight absorption.

INTRODUCTION

The origin of soil nitrogen has been highly controversial. A huge amount of nitrogen is removed from the arable lands every year through various processes such as harvest of crops, erosion, leaching and loss as elementary nitrogen from the soil surface (6,7). It is obvious, therefore, that in order to maintain their fertility, soils must have some mechanism by which they are able to fix nitrogen from some source and thus make up for the loss totally or partially. Various soil scientists have suggested different mechanisms to explain nitrogen fixation in the soils.

Sir John Rusell in a lecture in Bangalore (India) in 1935 stated that 'Legumes are the originators of soil nitrogen.' Because A. D. Hall in his book "Book of Rothamsted Experiments" reported the same thing in 1905, in Rothamsted, legumes are believed to be the orginators of soil nitrogen. On the other hand, F. E. Bear following the lead of Lohnis and Fred and others have declared that only five million tons of nitrogen are likely to be fixed by legumes in the whole world whilst 250 million tons of nitrogen are necessary for the world production of food, fodder and fibre.

Soils can also fix nitrogen by some free living nitrogen fixing organisms present in them. So far only three groups are recognised to have this power (1) facultative anaerobic bacteria of the Clostridia group (2) aerobic bacteria of the genus Azotobacter and (3) some blue green algae of the family Nostococeae. A considerable amount of work has been carried on recently on the part played by blue green algae in fixing atmospheric nitrogen, especially in paddy fields and thereby maintain soil fertility. De (2) Watanabe (22), Allen (1) Okuda and Yamaguchi (13) have reported considerable increase, even upto 21.8% (22) in the yield of paddy crop by the application of nitrogen fixing blue green algae to the paddy fields. Fritsch and De (9) and De (2) have concluded that nitrogen fixation in paddy soils

is purely through the agencies of alga and that the part played by bacteria is relatively unimportant. On the other hand, Uppal, Patel and Daji (21) have shown that Azotobactor plays an important role in the nitrogen recuperation of Vice soils at Karjat (India). Pfeiffer et al (15), Peterson (14) and others have expressed doubts about the economic importance of algae in soils, their views being largely based on the supposition that the algae make little growth except on the soil surface. Russell (17) has stated "There is no evidence yet that algae play any important role in enriehing the soils of temperate regions with nitrogen, although, species belonging to the geneva containing nitrogen fixing forms are fairly widespread. Gupta (10), Jaiswal (11) and Srivastava (19) have experimentally shown that algal contribution in enriching the land is very small in comparison to that of organic matter undergoing slow oxidation.

From the brief review given above it can be seen that no definite information is available regarding the amount of nitrogen fixed by blue green algae under ordinary conditions and the subject needs further study. Hence, in order to throw light on this problem, it was thought necessary to study quantitatively the part played by the algae in enriching our soils under normal aerobic conditions. Two species of blue-green algae, namely, Anabaena naviculoides and Tolypothrix tenuis and one species of green algae, Chlorella vulgaris, were inoculated in the soil and the increase in nitrogen after different intervals was compared with the increase in nitrogen observed when energy materials like wheat straw and sunnhemp mixed with soil were allowed to undergo slow oxidation both in presence and absence of Phosphates.

EXPERIMENTAL

The experiments were carried out with black cotton soil of India collected from the farm of the Agriculture College, Indore, M. P. (India) 250 gm. of soil after passing through 60 mesh sieve was taken in one lb, capacity glass bottles. To this, the required amounts of the organic materials, wheat straw (as 0.5% Carbon) and that of phosphates—Tata Basic Slag (as 0.25% P_2O_5) were added and the contents were thoroughly mixed. Then, wherever required, the soil in the bottles was inoculated with the two blue green algae namely, Anabaena naviculoides and Tolypothrix tenuis and with a green algae, Chlorella vulgaris. Two similar sets were arranged side by side, one of which was exposed to light under 500 watt electric bulb and the other was covered with a thick black cloth. In all the bottles the moisture content was maintained at 40% level throughout the experiment. At regular intervals of time, composite samples were taken out from all the bottles and were analysed for total carbon, total nitrogen and available P_2O_5. Pure unialgal cultures of the above three algae were obtained from Dr. A. K. Mitra, an eminent algologist and Head of the Algology Section, Botany Department, University of

Allahabad, Allahabad (India). Stock cultures of these algae were then prepared by growing them in the following media :—

Medium for Anabaena and Tolypothrix			Medium for Chlorella.		
KNO_3	...	0.2 gm.	NH_4NO_3	...	0.2 gm.
K_2HPO_4	...	0.2 gm.	K_2HPO_4	...	0.2 gm.
$MgSO_4.7H_2O$	...	0.2 gm.	$MgSO_4.7H_2O$	...	0.2 gm.
$Cacl_2$	...	0.1 gm.	$Ca\ Cl_2$	...	0.1 gm.
$FeCl_3$ (1%)	...	2 drops	$FeCl_3$ (1%)	...	2 drops
Pyrex distilled water		10 0 c.c.	Pyrex distilled water		1000 c.c.

Analysis of Original Soil, Organic Material and Basic Slag.

Estimation		Black Cotton Soil %	Wheat Straw %	Tata Basic glag (T. B. S.) %
Loss on ignition	...	...	89.9043	...
Ash	...	...	9.0941	...
HCl insoluble Silica	...	57.6600	5.0455	15.6846
Sesquioxide	...	28.9451	1.4168	...
Fe_2O_3	...	18.5650	0.6172	15.5650
Al_2O_3 (by difference)	...	10.3801	0.7996	5.4320
Total CaO	...	2.8464	0.8432	38.6946
Total MgO	...	1.0567	0.4081	4.8486
Total K_2O	...	0.8313	0.8014	0.6474
Total P_2O_5	...	0.1452	0.6036	7.1600
Available P_2O_5	...	0.0045	...	...
Total Carbon	...	0.4800	28.2650	...
Total Nitrogen	...	0.0458	0.6370	...
NH_3-N	...	0.0014	...	...
NO_3-N	...	0 0018	...	...
Calcium Carbonate	...	3.7500	...	..
C/N Ratio	...	10.62	60.07	..

TABLE No. 1

250 gm. Soil alone

LIGHT

Period of exposure in days.	Total Carbon (gm.)	Carbon Oxidised (gm)	Total Nitrogen (gm.)	Total Nitrogen Fixed (gm)	Avail. $P_2 O_5$ (gm.)	Efficiency.
0	1.200	—	0.1130	...	0.0112	...
60	1.122	0 078	0.1139	0.9	0.0119	11.5
120	1.059	0.141	0 1146	1.6	0.0123	11.1
180	1.036	0.164	0.1148	1.8	0.0125	10.4
240	1.021	0.179	0.1145	1.5	0.0126	...
DARK						
0	1.200	...	0.1130	...	0.0112	...
60	1.156	0.044	0.11337	0.37	0.0117	8.5
120	1.121	0.079	0.11364	0.64	0.0120	8.1
180	1.102	0.098	0.11378	0.78	0.0122	8.0
240	1.087	0.113	0.11360	0.60	0.0123	...

TABLE No. 2

250 gm. Soil+Anabaena

LIGHT

0	1.200	...	0.1130	...	0.0112	...
60	1.142	0.058	0.1144	1.4	0.0120	24.1
120	1.113	0.087	0.1150	2.0	0.0125	23.0
180	1.082	0.118	0.1153	2.3	0.0127	19.5
240	1.065	0.135	0.1149	1.9	0.0128	...
DARK						
0	1.200	...	0.1130	...	0.0112	...
60	1.58	0.042	0.11338	0.38	0.0118	9.0
120	1.22	0.078	0.11365	0.65	0.0121	8.3
180	1.105	0.095	0.11379	0.79	0.0124	8.3
240	1.090	0.110	0.11372	0.72	0.0125	...

TABLE No. 3

250 gm. Soil+Chlorella

LIGHT

Period of exposure in days.	Total Carbon in Gms.	Carbon oxidised (gms.)	Total Nitrogen (gms.)	Total Nitrogen fixed. (mgms.)	Available P_2O_5	Efficiency
0	1.200	...	0.1130	...	0.0112	...
60	1.146	0.054	0.1140	1.0	0.0121	18.5
120	1.122	0.078	0.1144	1.4	0.0127	17.9
180	1.094	0.106	0.1148	1.8	0.0130	17.0
240	1.079	0.121	0.1146	1.6	0.0131	...

DARK

Period of exposure in days.	Total Carbon in Gms.	Carbon oxidised (gms.)	Total Nitrogen (gms.)	Total Nitrogen fixed. (mgms.)	Available P_2O_5	Efficiency
0	1.200	...	0.1130	...	0.0112	...
60	1.158	0.042	0.11338	0.38	0.0119	9.0
120	1.124	0.076	0.11366	0.66	0.0122	8.7
180	1.106	0.094	0.11379	0.79	0.0124	8.4
240	1.091	0.109	0.11370	0.70	0.0125	...

TABLE No. 4

250 gm. Soil+Tolypothrix

LIGHT

0	1.200	...	0.1130	...	0.0112	...
60	1.143	0.057	0.1145	1.5	0.0120	26.3
120	1.116	0.084	0.1151	2.1	0.0126	25.0
180	1.087	0.113	0.1155	2.5	0.0128	22.1
240	1.070	0.130	0.1151	2.1	0.0129	...

DARK

0	1.200	...	0.1130	...	0.0112	...
60	1.157	0.043	0.11339	0.39	0.0119	9.1
120	1.123	0.077	0.11365	0.65	0.0121	8.4
180	1.106	0.094	0.11379	0.79	0.0123	8.4
240	1.090	0.110	0.11373	0.73	0.0124	...

TABLE No. 5

250 gms. Soil+0.25% P_2O_5 as T. B. S.

LIGHT

Period of exposure in days.	Total Carbon (gm.)	Carbon oxidised (gm.)	Total Nitrogen (gm.)	Total Nitrogen fixed (gm.)	Avail. P_2O_5 (gm.)	Efficiency
0	1.200	...	0.1130	...	0.3649	...
60	1.113	0.087	0.1141	1 1	0.3662	12.6
120	1.053	0.147	0.1148	1.8	0.3671	12.2
180	1.021	0.179	0.1151	2.1	0.3678	11.7
240	1.017	0.183	0.1146	1.6	0.3682	...
DARK						
0	1.200	...	0.1130	...	0.3649	...
60	1.150	0.050	0.11344	0.44	0.3658	8.8
120	1.113	0.087	0.11377	0.77	0.3665	8.7
180	1.099	0.106	0.11389	0.89	0.3670	8.4
240	1.076	0.124	0.11380	0.80	0.3673	...

TABLE No. 6

250 gm. Soil+0.25% P_2O_5 as T. B. S.+Anabaena.

LIGHT

0	1.200	...	0.1130	...	0.3649	...
60	1.140	0.060	0.1146	1.6	0.3663	26.7
120	1.112	0.088	0.1153	2.3	0.3673	26.1
180	1.077	0.123	0.1158	2.8	0.3681	22.8
240	1.058	0.142	0.1151	2.1	0.3684	...
DARK						
0	1.200	...	0.1130	...	0.3649	...
60	1.151	0.049	0.11346	0.46	0.3658	9.4
120	1 115	0.085	0.11379	0.79	0.3666	9.3
180	1.095	0.105	0.11390	0.90	0.3671	8.6
240	1.078	8.122	0.11384	0.84	0.3 74	...

TABLE No. 7

250 gm. Soil+0.25% P_2O_5 as T. B. S.+Chlorella

Period of exposure in days.	Total Carbon (gm.)	Carbon oxidised (gm.)	Total nitrogen (gm.)	Total nitrogen fixed (gm.)	Avail. P_2O_5	Efficiency.
LIGHT						
0	1.200	...	0.1130	...	0.3649	...
60	1.144	0.056	0.1142	1.2	0.3675	21.4
120	1.114	0.086	0.1148	1.8	0.3665	20.9
180	1.082	0.118	0.1152	2.2	0.3681	18.6
240	1.064	0.136	0.1148	1.8	0.3687	...
DARK						
0	1.200	...	0.1130	...	0.3649	...
60	1.152	0.048	0.11345	0.45	0.3656	9.4
120	1.115	0.085	0,11379	0.79	0.3666	9.3
180	1.096	0.104	0.11389	0.89	0.3673	8.6
240	1.080	0.120	0.11382	0.82	0.3674	...

TABLE No. 8

250 gm. Soil+0.25% P_2O_5 as T. B. S.+Tolypothrix

Period of exposure in days.	Total Carbon (gm.)	Carbon oxidised (gm.)	Total nitrogen (gm.)	Total nitrogen fixed (gm.)	Avail. P_2O_5	Efficiency.
LIGHT						
0	1.200	...	0.1130	...	0.3649	...
60	1.141	0.059	0.1147	1.7	0.3663	28.8
120	1.112	0.088	0.1154	2.4	0.3674	27.[illegible]
180	1.079	0.121	0.1160	3.0	0.3680	24.8
240	1.061	0.139	0.1154	2.4	0.3685	...
DARK						
0	1.200	...	0.1130	...	0.3649	...
60	1.152	0.048	0.11345	0.45	0.3657	9.4
120	1.113	0.087	0.11380	0.80	0.3666	9.2
180	1.095	0,105	0.11391	0.91	0.3672	8.7
240	1.079	0.121	0.11385	0.85	0.3674	...

TABLE No. 9

250 gm. Soil+0 5% Carbon as wheatstraw.

Period of exposure in days.	Total Carbon (gms.)	Carbon oxidised (gm.)	Total nitrogen (gm.)	Total nitrogen fixed (gms.)	Avail. P_2O_5 (mg.)	Efficiency.
LIGHT						
0	2.450	...	0.1438	...	0.0112	...
60	2.084	0.366	0.1517	7.9	0.0130	21.6
120	1.838	0.612	0.1556	11.8	0.0139	19.3
180	1.691	0.759	0.1579	14.1	0.0143	18.6
240	1.546	0.904	0.1561	12.3	0.0146	...
DARK						
0	2.450	...	0.1438	...	0.0112	...
60	2.248	0.202	0.1464	2.6	0.0124	12.9
120	2.074	0.376	0.1485	4.7	0.0130	12.5
180	1.992	0.458	0.1494	5.6	0.0133	12.2
240	1.938	0.512	0.1491	5.3	0.0134	...

TABLE No. 10

250 gm. Soil+0.5% Carbon as Wheat Straw+Anabaena.

LIGHT						
0	2 450	...	0.1438	...	0.0112	...
60	2.211	0.239	0.1543	10.5	0.0133	44.0
120	2.178	0.272	0.1598	16.0	0.0142	43.0
180	1.874	0.576	0.1640	20.2	0.0146	35.1
240	1.813	0.637	0.1615	17.7	0.0151	...
DARK						
0	2.450	...	0.1438	...	0.0112	...
60	2.251	0.199	0.1465	2.7	0.0125	13.5
120	2.076	0.374	0.1486	4.8	0.0133	12.8
180	1.994	0,456	0.1495	5.7	0.0134	12.5
240	1.940	0.510	0.1493	5.5	0.0135	...

TABLE No. 11

250 gm. Soil+0.5% Carbon as Wheat Straw+Chlorella.

LIGHT

Period of exposure in days.	Total Carbon (gm.)	Carbon oxidised (gm.)	Total nitrogen (gm.)	Total nitrogen fixed (gm.)	Avail. P_2O_5	Efficiency.
0	2.450	0.1438	0.1433	...	0.0112	...
60	2.215	0.1520	0.1520	8.2	0.0137	34.9
120	2.081	0.1558	0.1558	12.0	0.0143	32.5
180	1.8[illegible]3	0.567	0.1582	14.4	0.0152	26.0
240	1.821	0.629	0.1563	12.5	0.0155	...

DARK

0	2.450	...	0.1438	...	0.0112	...
60	2.252	0.193	0.1465	2.7	0.0126	13.6
120	2.078	0.372	0.1486	4.8	0.0132	12.9
180	1.985	0.465	0.1494	5.6	0.0135	12.3
240	1.941	0.509	0.1493	5.5	0.0137	...

TABLE No. 12

250 gm. Soil+0.5% Carbon as Wheat Straw+Tolypothrix.

LIGHT

Period of exposure in days.	Total Carbon (gm.)	Carbon oxidised (gm.)	Total nitrogen (gm.)	Total nitrogen fixed (gm.)	Avail. P_2O_5	Efficiency.
0	2.450	...	0.1438	...	0.0112	...
60	2.212	0.238	0-1549	11.1	0.0135	96.6
120	2.080	0.370	0.1604	16.6	0.0145	44.8
180	1.879	0.571	0.1647	20.9	0.0149	36 6
240	1.817	0.633	0.1624	18.6	0.0153	...

DARK

0	2.450	...	0.1438	...	0.0112	...
60	2.254	0.196	0.1465	2.7	0.0126	13.6
120	2.077	0.373	0.1486	4.3	0.0133	12.8
180	1.995	0.455	0.1495	5.7	0.0135	12.6
240	1.941	0.509	0.1493	5.5	0.0136	...

TABLE No. 13

250 gm. Soil+0.5% Carbon as Wheat Straw+0.25% P_2O_5 as T. B. S.

Period of exposure in days.	Total carbon (gm.)	Carbon oxidised (gm.)	Total nitrogen (gm.)	Total nitrogen fixed (gm.)	Avail. P_2O_5 (gm.)	Efficiency
LIGHT						
0	2.450	...	0.1438	...	0.3649	...
60	1.982	0.468	0.1620	18.2	0.3981	38.9
120	1.813	0.637	0.1674	23.6	0.4201	37.0
180	1.640	0.810	0.1704	26.6	0.4383	32.8
240	1.468	0.982	0.1682	24.4	0.4417	...
DARK						
0	2.450	...	0.1438	...	0.3649	...
60	2.203	0.247	0.1493	5.5	0.3901	22.3
120	2.054	0.396	0.1526	0.8	0.4115	22.0
180	1.953	0.497	0.1543	10.5	0.4277	21.1
240	1.887	0.563	0.1536	9.8	0.4300	...

TABLE No. 14

250 gm. Soil+0.5% Carbon as Wheat Straw+0.25% P_2O_5 as T. B. S.+Anabaena.

LIGHT						
0	2.450	...	0.1438	...	0.3649	...
60	2.112	0.338	0.1632	19.4	0.3989	57.4
120	1.942	0.508	0.1724	28.6	0.4209	55.2
180	1.763	0.687	0,1770	33.2	0.4387	48.3
240	1.719	0.731	0.1751	31.3	0.4420	...
DARK						
0	2.450	...	0.1438	...	0.3649	...
60	2.207	0.243	0.1495	5.7	0.3207	23.4
120	2.055	0.395	0.1526	8.8	0.4119	22.2
180	1.957	0.493	0.1545	10.7	0.4281	21.7
240	1.890	0.560	0-1539	10.1	0.4343	...

TABLE No. 15

250 gm. Soil+0.5% Carbon as Wheat Straw+0.25% P_2O_5 as T. B. S.+Chlorella

LIGHT

Period of exposure in days.	Total Carbon (gm.)	Carbon oxidised (gm.)	Total nitrogen (gm.)	Total nitrogen fixed (gm.)	Avail. P_2O_5 (gm.)	Efficiency
0	2.450	...	0.1438	...	0.3649	...
60	2.117	0.233	0.1625	18.7	0.3992	53.0
120	1.949	0.501	0.1676	23.8	0.4214	47.9
180	1.768	0.682	0.1708	27.0	0.4392	39.6
240	1.724	0.726	0.1688	25.0	0.4429	...
DARK						
0	2.450	...	0.1438	...	0.3649	...
60	2.209	0.241	0.1494	5.6	0.3906	23.2
120	2.056	0.394	0.1527	8.9	0.4121	22.6
180	1.960	0.490	0.1544	10.6	0.4282	21.6
240	1.892	0.558	0.1539	10.1	0.4345	...

TABLE No. 16

250 gm. Soil+0.5% Carbon as Wheat Straw+0.25% P_2O_5 as T. B. S.+Tolypothrix.

LLGHT

0	2.450	...	0.1438	...	0.3649	...
60	2.114	0.236	0.1637	19.9	0.3991	59.5
120	1.943	0.507	0.1727	28.9	0,4212	57.0
180	1.765	0.685	0.1781	34.3	0.4389	50.1
240	1.721	0.729	0.1765	32.7	0.4426	...
DARK						
0	2.450	...	0.1438	...	0.3649	...
60	2.206	0.244	0.1495	5.7	0.3906	23.3
120	2.056	0.394	0.1527	8.9	0.4231	21.8
240	1.888	0.562	0.1537	9.9	0.4344	...

DISCUSSION

A careful examination of the results recorded in previous pages shows that when soil organic matter undergoes slow oxidation in the presence of air and moisture, there is a small increase in the nitrogen content of the system. Both oxidation of carbon and fixation of nitrogen are enhanced by the addition of the phosphate, Tata basic slag, and are always found to be greater in light than in the dark. Dhar et al (6, 7), after making a systematic study of the problem of nitrogen fixation have observed a small increase in nitrogen without added organic matter in Indian soils rich in humus and calcium phosphate. Schneider (18) Remy (16), Mockeridge (12), Zoond (24), de Rossi (5) and many others have found a gain in nitrogen by incubating soils for several hours.

As a result of mixing the energy material, wheat straw, in soil both in persence and absence of phosphates, there was a further increase more in the phosphated than in the unphosphated one, in the carbon oxidation and nitrogon fixation in the system.

In sets inoculated with algae some saving in carbon and a small increase in nitrogen was always found in light, though in dark, there was no such significant difference in the carbon and nitrogen status of the systems. The order of carbon saving was Anabena $<$ Tolypothrix $<$ Chlorella. It is interesting to note that the saving of carbon in sets inoculated with chlorella is greater than that in sets inoculated either with Anabaena or Tolypothrix. This is in agreement with the observation of William and Burris (23) and others that the growth rates of blue green algae are much smaller than those for chlorella.

In sets inoculated with chlorella there is a slightly greater increase in nitrogen than those of the uninoculated ones. It is interesting to note that though chlorella is not a nitrogen fixer, even then under our experimental conditions, it shows a small fixation of nitrogen. The probable reason of this seems to be that in soils Chlorella lives in symbiosis with azotobacter (17). During this process it supplied the bacteria with carbohydrates and the bacteria in turn fixes nitrogon. In sets inoculated with Tolypothrix and Anabena there is appreciable increase in the nitrogen content over that of the control sets, the increase being greater in the case of Tolypothrix than that of Anabaena. This is in agreement with the general observations made by various algologists that Tolypothrix is a better fixer of nitrogen than Anabaena.

It is of interest to note that in 180 days the increase in nitrogen over the initial nitrogen of the system due to the inoculation of Anabaena and Tolypothrix is 2.3 and 2.5 mg. which is smal and almost negligible in comparison to that fixed by the oxidation of organic material and phosphates, the value being 26.6 mg. for wheatstraw in the presence of Tata basic slag.

Another important observation is that the influence of algae is more pronounced in sets containing the energy materials and the phosphate showing that these materials activate the growth of algae. The increase in nitrogen in 180 days, due to Anabaena and Tolypothrix when inoculated in soil alone is only 0.5 and 0.7 mg. respectively over the control while in presence of wheat straw, the respective increase was 6.1 and 6.8 mg. In presence of the phosphate, Tata basic slag, these vales are slightly higher. These results are in agreement with the observations of De and Sulaiman (4), and De and Mandal (3), who reported that nitrogen fixation

by blue green algae is greater in presenee of crops than in their absence. They attributed this stimulating effect to the increased supply of carbon dioxide resulting from the respiration and decomposition of the roots of higher plants. An increase in algal activity by the application of 60 lbs. P_2O_5 per acre and by liming in lowland soils was reported by Fernandez (8).

Furthermore, the experimental results show that the efficiency of nitrogen fixation, which is defined as the number of milligrams of nitrogen fixed per gram of carbon oxidised, is always found to be much greater in sets inoculated with alge than those without it. With Anabaena and Tolypothrix the efficiency is always found to be greater than that with Chlorella. This is because along with carbon saving, both Anabaena and Tolypothrix being nitrogen fixers, are able to increase the nitrogen content of the system, thus giving a higher efficiency.

Another very important observation is that the available phosphate of the system increases with the oxidation of carbon and the increase is greater in the inoculated sets than that in the uninoculated ones. The incrcase in available phosphate by the inoculation of algae may be due to the fact that algae excrete some organic acids which might dissolve some of the phosphate. In addition, the organic phosphorus of the nucleic acids and nucleotides, etc....of the algal material, may be made available by dephosphorrylation (20).

It is, therefore, concluded from our investigations that though algae can fix some atmospheric nitrogen in the soil, the amount of nitrogen fixed by algae is small and almost negligible in comparison to that fixed by organic materials undergoing slow oxidation in the presence of light. Similar observations have been made by Dhar et al (6, 10, 11, 19) previously. These results further prove that, as suggested by Dhar et al, the major source of soil nitrogen is the organic matter, which, in addition to the nitrogen present in it, also fixes atmospheric nitrogen while undergoing solw oxidation at the soil surface.

REFERENCES

1. Allen, M. B., Photosynthetic nitrogen fixation by Blue Green Algae (1955).
2. De, P. K., *Proc. Roy. Soc. London*, *B*, 127 : 121 (1939).
3. De, P. K. & Mandal L. N., *Soil Science*, 81 :453 (1956).
4. De, P. K. & Sulaiman, M., *Ind. J. Agric. Sci.* 22 : 375 (1942).
5. De, Rossi, G., Les microbes du sol et la fixation de lazote atmospheric, *Soc. Intern. Microbiol. Bult., Italy*, 4 : 418-483 (1932).
6. Dhar, N. R., *Presi. Addr. Nat. Acad. Sci. India*, 47 (1952).
7. Dhar, N. R., Influence of light on some biochemical proeesses pp. 33-34 (1935).
8. Fernandez, S. C., *J. Soil. Sci. Soc. Phili.* pp. 18 : 19-22 (1956).
9. Fritsch, F. E. and De, P. K., *Nature*, 142 : 878 (1938).
10. Gupta, G. P., *D. Phil. Thesis Alld. University*, (1954).
11. Jaiswal, S. P., *D. Phil. Thesis Alld. University* (1964).
12. Mockeridge, F. A., *Proc. Roy. Soc. London.* B. 89 : 508-533 (1917).
13. Okhuda, A., and Yamaguchi, M., *Mem. Res Ints. Food. Sci. Kyoto University*, 4 : 1 (1952).

14. Peterson, J. B., *Dansk, Bot. Ark.* K. 8, 1 (1935).

15. Pfeiffer, T. and Guttman, *M. H. London Inst. Uni. Breshau* 5 : 657-713.(1910).

16. Remy, T., Untersuchungen Uber die sticksotoffs Ungsvorgauge in ther Beichung Zum. *Boden, Klima cent Bakt.* II., 22 : 561-651 (1909).

17. Russell, E. J., Soil Conditions and Plant Growth (1950).

18. Schneider, P., *Studien Uber die stickstoff samluag in Ackeroboden Landw Jb.*, 35 Eng. Bd. IV, 62-68 (1906).

19. Srivastava, G. P., *D. Phil. Thesis, All. University* (1964).

20. Thompson, L. M. and Black, C. A., *Soil Sci. Soc. Amer. Proc.* 14 : 147 (1950).

21. Uppal, B. N., Patel, M. K., *Ind. J. Agric. Sc.*, 9 : 689 (1939).

22. Watanabe, A., *Bot. Mag. Tokyo*, Vol. 69 : No. 820-21 (1956).

23. William, A. E. and Burris, R. H., *Amer. J. Bot.* Vol. 39 : 340-42 (1952).

24. Zoond, A., *Brit. J. Exp. Biol.*, 4 : 105-113 (1926).

RETARDING INFLUENCE OF ALGAE ON NITROGEN LOSS FROM BLACK COTTON SOILS TREATED WITH NITROGENOUS FERTILIZERS

By

N. R. DHAR *and* P. G. DEO

Sheila Dhar Institute of Soil Science, University of Allahabad

ABSTRACT

World agriculture shows that the recovery of nitrogen from fertilizers and manures is not high and usually varies from 25% to 30%.

Dhar and co-workers have advanced the view that in the nitrification of all nitrogenous compounds applied to land, the unstable and explosive substance, ammonium nitrite is formed and it breaks up into nitrogen gas and water with much heat evolution ($NH_4NO_2 = N_2 + 2H_2O + 718$ K. Cal.). This phenomenon leads to the loss of 60 to 70% of nitrogen applied as nitrogen gas without benefitting the soil or the crop. This loss can be checked and retarded by adding organic substances like straw, leaves, powdered coal, etc. Experiments carried on with black cotton soils show that the algae, Chlorella, Tolypothrix and Anabaena can retard the loss from the soil on the application of ammonium sulphate more in presence of organic matter and phosphate than in their absence. Wheatstraw fortified with phosphates retards nitrogen loss much more than the algae.

Nitrogen being an elusive substance, the nitrogen fixed or added as organic or inorganic fertilizers does not remain in the soil for a long time under ordinary conditions; specially the inorganic fertilizers like ammonium sulphate, sodium nitrate, etc. suffer severe losses from the soil. It has been observed that a major part of nitrogen added in the form of artificial fertilizers is lost from the soil in a short time and because of this fact the recovery of nitrogen by crops is not high. Löhnis and Fred (15) have reported the following recovery of the three major plant nutrients from their field experiments.

Nitrogen (%)	Phosphorus (%)	Potassium (%)
7.8 to 46.1	10.1 to 75.6	22.4 to 85.1

It is clear from the above data of the plant nutrients—nitrogen, phosphorus and potassium—the recovery of nitrogen by crops is the lowest.

Snyder (19) working on Minnesota soils, Swanson and Gainey (14) on Kansas soils and Bracken and Greaves (2) on Utaw soils have reported from their experiments that only one third of the lost nitrogen was recovered in the crop. Lyon (17) and Wilson (23) have also recorded loss of nitrogen from similar experiments. Megitt (18) has also reported huge losses of nitrogen in humid soils of Assam. Bizell (1) observed that one-third of the nitrogen added to the soils as ammonium sulphate or sodium nitrate is lost when timothy and market garden crops are grown.

Dhar et al (5-9) have carried on systematic work on the process of ammonification and nitrification. Dhar, Tandon and Mukherji (12) and Dhar and Mukherji (10) have demonstrated that during the oxidation and nitrification of ammonium salts nitrogen is always lost in the gaseous state. Dhar and Pant (11) have reported considerable losses of nitrogen in soils and oxide surfaces during the decomposition of urea and gelatine in the complete absence of micro-organisms. Dhar (5-7), therefore, concluded from these observations that these processes of ammonification and nitrification are partly photochemical and chemical. He suggested that the loss of nitrogen during these processes is due to the formation of a very unstable substance, ammonium nitrite, which decomposes very easily producing nitrogen gas and water with marked evolution of heat according to the following equation ;—

$$NH_4NO_2 \longrightarrow N_2 + 2H_2O - 718 \text{ K. Cal.}$$

How to check nitrogen loss from the soil has, therefore remained a problem for the soil scientists and various attempts were made to find out some method of checking the loss of soil or added nitrogen. Of the various methods used in this connection, the application of organic matter has been found to be the most beneficial. Dhar and his coworkers (7-12) have carried on a large number of experiments for determining the retarding influence of various organic materials on nitrogen loss from the soils both in presence and absence of phosphates. Their results have conclusively proved that the carbonaceous substance like straw, molasses, glucose, starch, etc. preserve the soil or added nitrogen by decreasing the velocity of oxidation of the nitrogenous compounds present in the system and thus decreasing the formation and subsequent decomposition of ammonium nitrite.

The part played by the blue green alage in nitrogen fixation has been studied by many workers (13.22) but very few could realize their importance in checking nitrogen loss from the soils. Gautier and Drouin (15) exposed samples of artificial soil free from organic materials and containing only ammoniacal nitrogen in a sheltered position for a long time, the soil became, in course of time covered with algae. This resulted in a loss in total nitrogen, a still greater loss in ammoniacal nitrogen and a gain in organic nitrogen ; the ammonia nitrogen was converted into organic nitrogen by the algae. With an increase in growth there was a decrease in the loss of total nitrogen. These workers, therefore, reported that algae also play a part in preventing the loss of ammonia nitrogen, as well as the leaching out of nitrates from the soil. The fact that the fixation of atmospheric nigtrogen by algae is mostly suppressed when they are grown in a medium containing combined nitrogen suggests the preferential utilisation by algae of the nitrogen in the system to that in the atomosphere. The nitrogen of the system which is utilized by the algae in building up its body proteins is temporarily saved from being lost to the atmoshpere.

It is clear from the brief review given above that algae do play some part in checking nitrogen loss from the soils. To throw further light on this problem of nitrogen loss and its retardation by algae, we have carried on many experiments by inoculating the two blue green algae, Anabaena naviculoides and Tolypothrix tenuis and a green alga, chlorella vulgaris in black cotton soil to which ammonium sulphate was added both in presence and absence of wheat straw and Tata basic slag.

EXPERIMENTAL

Black cotton soil of India, collected from Government Agriculture College Farm, Indore (India) was used in the present experiment. 250 gm. of soil after passing through 60 mesh sieve was taken in 1 lb. capacity glass bottles. To this. required amounts of the organic material, wheat-straw (as 0·5% carbon) and of phosphate, Tata basie slag (as 0·25% P_2O_5) and the nitrogenous fertilizer, ammonium sulphate (as 0·1% nitrogen), were added and the contents were throughly mixed. When required, the soil in the bottles was inoculated with the two blue green algae, Anabaena naviculoides and Tolypothrix tenuis and a green alga, Chlorella vulgaris. Two similar sets were arranged, out of which one was exposed to light under 500 watt bulb and the other was covered with a thick black cloth. In all the bottles the moisture was maintained at 40% level throughout the experimental period. At regular intervals of time, composite samples were taken out from each bottle and were analyzed for total carbon, total nitrogen ammonia and nitrate nitrogen.

Bacteria-free unialgal cultures of the above three alagae were obtained from Dr. A. K. Mitra, an eminent algologist. Stock cultures of these algae were then prepared by growing them in the following Media :—

Medium for Anabaena and Tolypothrix		Medium for Chlorella	
KNO_3	... 0·2 gm.	NH_4NO_3	... 0·2 gm.
K_2HPO_4	... 0·2 gm.	K_2HPO_4	... 0·2 gm.
$MgSO_4 . 7H_2O$	... 0·2 gm.	$MgSO_4 . 7H_2O$	... 0·2 gm.
$CaCl_2$	... 0·1 gm.	$CaCl_2$	... 0.1 gm.
$FeCl_3$ (1%)	... 2 drops.	$FeCl_3$ (1%)	... 2 drops.
Pyrex distilled water 1000 cc.		Pyrex distilled water 1000 cc.	

Analysis of Original Soil, Wheat Straw and Tata Basic Slag.

Estimation	Black Cotton Soil (%)	Wheat-straw (%)	Tata Basic Slag. (%)
Loss on ignition	...	89·9043	...
Ash	...	9·0941	...
HCl insoluble silica	57·6600	5·0455	15·6846
Sesquioxide	28·9451	1·4168	...
Fe_2O_3	18·5650	0·6172	15·5650
Al_2O_3 (by difference)	10·3801	0·7996	5·4320
Total CaO	2·8464	0·8432	38·6946
Total MgO	1·0567	0·4081	4·8486
Total K_2O	0·8313	0·8014	0·6474
Total P_2O_5	0·1452	0·6036	7·1600
Avl. P_2O_5	0·0045	...	4·0520
Total Carbon	0·4800	38·2650	...
Total Nitrogen	0·0458	0·6370	...
NH_3-N	0·0014	...	...
NO_3-N	0·0018	...	...
Calcium carbonate	3·7500	...	...
C/Nratio	10·62	60·07	...

Note—T. B. S.=Tata Basic Slag.

TABLE 1

250 gm. Soil +0·1% nitrogen as Ammonium sulphate

LIGHT

Period of exposure in days	Total Carbon (gm.)	Total nitrogen (gm.)	Total nitrogen lost (gm.)	NH_3–N (gm)	NO_3—N (gm)	Total Avl. nitrogen (gm.)
0	1·200	0·3630	...	0·2535	0·0045	0·2580
60	1·114	0·1810	50·1	0·1149	0·0144	0·1293
120	1·048	0·1372	62·2	0·0622	0·0322	0·0944
180	1·016	0·1076	70·4	0·02[illegible]1	0·0375	0·0666
240	0·992	0·0944	74·0	0·0202	0·0393	0·0595
DARK						
0	1·200	0·3630	...	0·2535	0·0045	0·2530
60	1·148	0·2178	40·0	0·1469	0·0117	0·1580
120	1·114	0·1831	49·6	0·1111	0·0212	0·1323
180	1·094	0·1592	56·1	0·0897	0·0251	0·1148
240	1·077	10·1434	60·5	0·0735	0·0300	0·1035

TABLE 2

250 gm. Soil + 0·1% nitrogen as Ammonium sulphate + Chlorella.

LIGHT

0	1·200	0·3630	...	0·2535	0·0045	0·2580
60	1·143	0·1913	47·3	0·1127	0·0128	0·1255
120	1·117	0·1561	57·0	0·0598	0·0296	0·0894
180	1·089	0·1300	64·2	0·0262	0·0365	0·0627
240	1·070	0·1082	70·2	0·0166	0·0383	0·0547
DARK						
0	1·200	0·3630	...	0·2535	0·0045	0·2580
60	1·150	0·2252	38·2	0·1466	0·0117	0·1583
120	1·118	0·1907	47·5	0·1104	0·0213	0·1317
180	1·100	0·1692	53·4	0·0893	0·0250	0·1143
240	1·081	0·1510	58·4	0·0731	0·0298	0·1029

TABLE 3

250 gm. soil $+0\cdot1\%$ nitrogen as Ammonium sulphate + Anabaena

LIGHT

Period of exposure in days	Totol Carbon (gm.)	Total nitrogen (gm.)	Total nitrogen lost (%)	NH_3-N (gm.)	NO_3-N (gm.)	Total avail nitrogen (gm.)
0	1·200	0·3630	...	0·2535	0·0095	0·2580
60	1·139	0·1844	49·2	0·1145	0·0123	0·1268
120	1·109	0·1278	59·3	0·0632	0·0276	0·0908
180	1·075	0·1209	66·7	0·0298	0·0347	0·0645
240	1·054	0·1013	72·1	0·0197	0·0371	0·0568
DARK						
0	1·200	0·3630	...	0·2535	0·0045	0·2580
60	1·150	0·2221	38·8	0·1468	0·0116	0·1514
120	1·117	0·1874	48·4	0·1109	0·0211	0·1320
180	1·098	0·1649	54·6	0·0896	0·0250	0·1146
240	1·080	0·1492	58·9	0·0734	0·0297	0·1031

TABLE 4

250 gm. soil + 0·1 % nitrogen as Ammonium sulphate + Tolypothrix

LIGHT

0	1·200	0·3630	...	0·2535	0·0045	0·2580
60	1·141	0·1862	48·7	0·1148	0·0113	0·1261
120	1·111	0·1507	58·5	0·0639	0·0262	0·0901
180	1·082	0·1242	65·8	0·0307	0·0331	0·0638
240	1·060	0·1038	71·4	0·0203	0·0357	0·0568
DARK						
0	1·200	0·3630	...	0·2535	0·0045	0·2580
60	1·149	0·2229	38·6	0·1469	0·0115	0·1580
120	1·118	0·1884	48·1	0·1110	0·0209	0·1319
180	1·099	0·1967	54·0	0·0898	0·0246	0·1144
240	1·079	0·1612	55·6	0·0735	0·0295	0·1030

TABLE 5

250 gm. soil + 0·25% P_2O_5 as T. B. S. + 0·1% Nitrogen as Ammonium Sulphate

LIGHT

Period of exposure in days	Total Carbon (gm.)	Total nitrogen (gm.)	Total nitrogen lost (%)	NH_3—N	NO_3—N	Total avail. nitrogen (gm.)
0	1·200	0·3630	...	0·2535	0·0045	0·2580
60	1·109	0·1895	47·8	0·1205	0·0169	0·1374
120	1·044	0·1536	59·0	0·0687	0·0316	0·1003
180	1·013	0·1242	65·8	0·0384	0·0402	0·0786
240	0·984	0·1027	71·7	0·0235	0·0429	0·0664

DARK

0	1·200	0·3630	...	0·2535	0·0045	0·2580
60	1·141	0·2399	33·9	0·1596	0·0132	0·1728
120	1·106	0·2098	42·2	0·1275	0·0254	0·1529
180	1·085	0·1913	47·3	0·1115	0·0286	0·1401
240	1·066	0·1742	52·0	0·0920	0·0308	0·1218

TABLE 6

250 gm. soil + 0·25% P_2O_5 as T. B. S. + 0·1% Nitrogen as Ammonium Sulphate

LIGHT

0	1·200	0·3630	...	0·2535	0·0045	0·2580
60	1·141	0·2011	54·6	0·1169	0·0152	0·1321
120	1·112	0·1605	55·8	0·0643	0·0307	0·0950
180	1·076	0·1369	62·3	0·0331	0·0393	0·0724
240	1·051	0·1227	66·2	0·0195	0·0409	0·0604

DARK

0	1·200	0·3630	...	0·2535	0·0045	0·2580
60	1·145	0·2461	32·2	0·1593	0·0131	0·1724
120	1·110	0·2150	40·8	0·1269	0·0255	0·1524
180	1·090	0·1963	45·8	0·1113	0·0283	0·1396
240	1·071	0·1801	50·4	0·0905	0·0308	0·1213

TABLE 7

250 gm. Soil + 0·25% P_2O_5 as T. B. S. + 0·1% nitrogen as Ammonium sulphate + Anabaena

LIGHT

Period of exposure in days	Total Carbon (gm.)	Total Nitrogen (gm.)	Total nitrogen lost (%)	NH_3—N	NO_3—N	Total avail. nitrogen (gm.)
0	1·200	0·3630	...	0·2535	0·0045	0·2580
60	1·136	0·1957	46·1	0·1198	0·0146	0·1344
120	1·108	0·1543	57·5	0·0678	0·0300	0·0976
180	1·071	0·1285	64·6	0·0375	0·0383	0·0758
240	1·047	0·1139	68·6	0·0228	0·0401	0·0629
DARK						
0	1·200	0·3630	...	0·2535	0·0045	0.2580
60	1·144	0·2445	32·6	0·1596	0·0130	0·1726
120	1·108	0·2118	41·6	0·1271	0·0253	0·1525
180	1·087	0·1931	46·8	0·1115	0·0283	0·1396
240	1·069	0·1757	51·6	0·0905	0·0306	0·1215

TABLE 8

250 gm. soil + 0·25% P_2O_5 as T. B. S. + 0·1% nitrogen as Ammonium sulphate + Tolypothrix

LIGHT

0	1·200	0·3630	—	0·2535	0·0045	0·2580
60	1·138	0·1968	45·8	0·1200	0·0137	0·1337
120	1·109	0·1583	56·4	0·0681	0·0284	0·0965
180	1·073	0·1318	63·7	0·0386	0·0357	0·0743
240	1·048	0·1183	67·4	0·0234	0·0383	0·0617
DARK						
0	1·200	0·3630	...	0·2535	0·0045	0·2580
60	1·146	0·2454	32·4	0·1596	0·0128	0·1724
120	1·108	0·2131	41·3	0·1273	0·0250	0·1523
180	1·088	0·1957	46·1	0·1115	0·0283	0·1398
240	1·070	0·1783	50·9	0·0910	0·0303	0·1213

TABLE 9

250 gm. soil + 0·5% carbon as wheat straw + 0·1% nitrogen as ammonium sulphate

LIGHT

Period of exposure in days	Total carbon (gm.)	Total nitrogen (gm.)	Total nitrogen lost (%)	NH_3-N (gm.)	NO_3-N (gm.)	Total avail. nitrogen (gm.)
0	2·450	0·3938	...	0·2535	0·0045	0·2580
60	2·059	0·2406	38·9	0·1478	0·0230	0·1708
120	1·802	0·2020	48·6	0·0860	0·0443	0·1303
240	1·523	0·1638	58·4	0·0453	0·0663	0·1116

DARK

0	2·450	0·3938	...	0·2535	0·0045	0·2580
60	2·231	0·2692	31·5	0·1766	0·0149	0·1915
120	2·056	0·2343	40·5	0·1453	0·0261	0·1724
180	1·975	0·2172	44·8	0·1320	0·0312	0·1632
240	1·889	0·2005	49·1	0·1187	0·0354	0·1541

TABLE 10

250 gm. soil + 0·5% carbon as wheat straw + 0·1% nitrogen as ammonium sulphate + chlorella

LIGHT

0	2·450	0·3938	...	0·2535	00·045	0·2580
60	2·234	0·2642	32·9	0·1382	0·0196	0·1578
120	2·056	0·2225	43·5	0·0794	0·0432	0·1226
180	1·857	0·2017	48·8	0·0494	0·0597	0·1096
240	1·733	0·1859	52·8	0·0404	0·0644	0·1048

DARK

0	2·450	0·3938	...	0·2535	0·0045	0·2580
60	2·234	0·2724	30·8	0·1759	0·0145	0·1904
120	2·062	0·2418	38·6	0·1447	0·0260	0·1707
180	1·979	0·2224	43·5	0·1313	0·0309	0·1622
240	1·893	0·2071	48·4	0·1181	0·0352	0·1533

TABLE 11

250 gm. soil + 0·5% carbon as wheat straw + 0·1% nitrongen as ammonium sulphate + Anabaena

LIGHT

Period of exposure in days	Total carbon (gm.)	Total nitrogen (gm.)	Total nitrogen lost (gm.)	NH_3-N	NO_3—N	Total avail. nitrogen (gm.)
0	2·450	0·3938	...	0·2535	0·0045	0·2580
60	2·186	0·2461	37·4	0·1437	0·0180	0·1617
120	2·048	0·2087	47·0	0·0848	5·0425	0·1273
180	1·845	0·1915	51·3	0·0534	0·0584	0·1118
240	1·722	0·1741	55·8	0·0442	0·0631	0·1073
DARK						
0	2·450	0·3938	...	0·2535	0·0045	0·2580
60	2·234	0·2712	31·1	0·1763	0·0144	0·1907
120	2·060	0·2358	40·1	0·1451	0·0258	0·1709
180	1·978	0·2187	44·4	0·1317	0·0308	0·1625
240	1·891	0·2016	48·8	0·1186	0·0349	0·1535

TABLB 12

250 gm. soil + 0·5% carbon as wheat straw + 0·1% nitrogen as ammonium sulphate + Tolypothrix

LIGHT

0	2·450	0·3938	...	0·2535	0·0045	0·2580
60	2·189	0·2544	35·4	0·1445	0·0156	0·1601
120	2·051	0·2134	45·8	0·0853	0·0401	0·1254
180	1·894	0·1949	50·5	0·0541	0·0568	0·1109
240	1·727	0·1776	54·9	0·0452	0·0608	0·1060
DARK						
0	2·450	0·3938	...	0·2535	0·0045	0·2580
60	2·233	0.2717	31·0	0·1764	0·0143	0·1907
120	2·061	0·2387	39·4	0·1453	0 0255	0·1708
180	1·980	0 2205	44·0	0·1318	0·0305	0·1623
240	1·891	0·2044	48·1	0·1186	0·0349	0·1535

TABLE 13

250 gm. soil + 0·5% carbon as wheat straw + 0·25% P_2O_5 as T. B. S. + 0·1% nitrogen as ammonium sulphate

LIGHT

Period of exposure in days	Total carbon (gm.)	Total nitrogen (gm.)	Total nitrogen lost (%)	NH_3—N	NO_3—N	Total avail. nitrogen (gm.)
0	2·450	0·3938	...	0·2535	0·0045	0·2580
60	1·957	0·2525	35·8	0·1566	0·0248	0·1814
120	1·786	0·2156	45·2	0·0954	0·0607	0·1561
180	1·614	0·1953	50·4	0·0614	0·0821	0·1435
240	1·494	0·1816	53·9	0·0475	0·0889	0·1369

DARK

0	2·450	0·3938	...	0·2535	0·0045	0·2580
60	2·192	0·2794	29·0	0·1796	0·0162	0·1958
120	2·035	0·2403	39·0	0·1485	0·0275	0·1760
180	1·940	0·2271	42·6	0·1361	0·0325	0·1689
240	1·856	0·2075	47·3	0·1207	0·0385	0·1592

TABLE 14

250 gm. soil + 0·5% carbon as wheat straw + 0·25% P_2O_5 as T. B. S. +0·1% nitrogen as Ammonium sulphate + Chlorella

LIGHT

0	2·450	0·3938	...	0·2535	0·0045	0·2580
60	2·094	0·2644	32·8	0·1494	0·0210	0·1704
120	1·921	0·2388	39·3	0·0862	6·0587	0·1449
180	1·746	0·2187	44·5	0·0527	0·0813	0·1340
240	1·626	0·2056	47·8	0·0401	0·0897	0·1298

DARK

0	2·450	0·3938	...	0·2535	0·0045	0·2580
60	2·197	0·2807	28·7	0·1793	0·0159	0·1952
120	2·039	0·2458	37·6	0·1473	0·0274	0·1752
180	1·942	0·2356	40·2	0·1356	0·0325	0·1681
240	1·860	0·2142	45·6	0·1203	0·0383	0·1586

TABLE 15

250 gm. soil + 0·1% carbon as wheat straw + 0·25% P_2O_5 as T. B. S. as ammonium sulphate + Anabaena

LIGHT

Period of exposure in days	Total carbon (gm.)	Total nitrogen (gm.)	Total nitrogen lost (gm.)	NH_3-N	NO_3-N	Total avail. nitrogen (gm.)
0	2·450	0·3938	.	0·2535	0·0045	0·2580
60	2·086	0·2577	34·5	0·1525	0·0201	0·1726
120	1·913	0·2324	41·0	0·0918	0·0565	0·1483
180	1·734	0·2063	47·6	0·0580	0·0798	0·1378
240	1·611	0·1953	50·4	0·0496	0·0885	0·1331

DARK

Period of exposure in days	Total carbon (gm.)	Total nitrogen (gm.)	Total nitrogen lost (gm.)	NH_3-N	NO_3-N	Total avail. nitrogen (gm.)
0	2·450	6·3938	...	0·2535	0·0045	0·2580
60	2·196	0·2801	28·8	0·1795	0·0158	0·1953
120	2·038	0·2420	38·3	0·1482	0·0272	0·1754
180	1·943	0·2287	42·9	0·1360	0·0324	0·1684
240	1·860	0·2103	46·6	0 1205	0·0383	0·1588

TABLE 16

250 gm. soil + 0·1% carbon as wheat straw 0·25% as T. B. S. + 0·1% nitrogen as ammonium sulphate + Tolypothrix

LIGHT

0	2·450	0·3938	...	0·2535	0·0045	0·2580
60	2·089	0·2586	33·4	0·1532	0 0181	0·1713
120	1·917	0·2339	40·6	0·0926	0·0539	0·1465
180	1·738	0·2119	46·2	0·0589	0·0778	0·1367
240	1·618	0·2008	49·0	0·0458	0·0861	0·1319

DARK

0	2·450	0 3938	...	0·2535	0·0045	0·2580
60	2·196	0·2812	28·6	0·1796	0·0157	0·1953
120	2·039	0·2442	38·0	0·1448	0·0268	0·1752
180	1·941	0·2316	41·2	0·1360	0·0323	0·1683
240	1·861	0·2111	45·9	0·1205	0·0382	0·1587

DISCUSSION

A perusal of the experimental results recorded in the previous pages show that in all sets there is a marked loss of nitrogen. The loss of nitrogen is always greater In sets exposed to light than that in corresponding sets kept in dark showing that light enhances the rate of nitrogen loss. Similar observations have been made by Dhar, Tondon and Mukherjee (12) and Dhar and Mukekherji (10). These observations on nitrogen loss in presence of air recorded in the previous pages, which are unaccountable from the bacterial viewpoint, become clear from the following considerations.

The soil contains some ammonium salts mainly as a result of the oxidation of proteins and amino acids present therein. In the p·occss of nitrification caused by light absorption or bacterial action the ammonium salts are first oxidized to nitrite. In other words, ammonium nitrite may be produced in the soil when a supply of air and light or nitrifying bacteria is available. Some years ago Dhar *et al* (10,11) observed that solutious of ammonium nitrite decompose into nitrogen and water when exposed to sunlight and this photochemical decomposition is facilitated by acids and different solid surfaces. The experiments of Dhar *et al* by exposing solutions of ammonium salts and sodium nitrite to sunlight, mixed with sterilized or unsterilized soil, showed marked decomposition of ammonium nitrite in light. In the soil the process of ammonification and nitrification go on simultaneously and thus at a certain stage in the process of oxidation, ammonium nitrite may be generated and this being an unstable substance, specially in the presence of light and the soil surface acting as a catalyst, partially decomposes with the liberation of gaseous nitrogen according to the following equation :—

$$NH_4NO_2 \rightarrow N_2 + 2H_2O + 718 \text{ K. Cal.}$$

A very interesting observation is that the loss of nitrogen is always smaller in sets containing the organic material wheat straw than in the corresponding sets without wheat straw showing that the straw retards the nitrogen loss. It is well know that the soil nitrogen cannot be increased without a corresponding increase in its total carbon content. This naturally leads us to the conclusion that in the presence of carbonaceous matter the soil nitrogen is fixed and protected. Dhar (5,6) has suggested that the carbonaceous matter acts as a negative catalyst and retards the process of nitrification which is an oxidation reaction. Unless nitrification takes place and nitrite ion in formed in place of ammonium ion, the formation and decomposition of ammonium nitrite are not possible. Hence in presence of large amounts of carbonaceous matter this type of loss of nitrogen would not be prononounced.

In several publications from this laboratory it has been shown that both carbohydrates and fats markedly act as negative catalysts and retard the oxidation of amino acids and proteins and that is why carbohydrates and fats act as protein sharer in the animal body. Similarly in the soil carbonaceous substances like cellulose, lignin, fats etc. present in the organic matter retard the oxidation of proteins, amino acids and ammonium salts by air and thus act as protectors of soil nitrogenous compounds. It has been also pointed out by Dhar *et al* that in the presence of organic materials, along with the retardation of the oxidation of ammonium salts, atmospheric nitrogen fixation takes place and thus the apparent loss of nitrogen becomes smaller.

It is of interest to record that in our experiments the sets inoculated with algae there is always some saving in the carbon oxidation and in total nitrogen loss

although the available nitrogen is greater in the uninoculated sets than in the inoculated sets. This shows that algae, during their growth, absorb ammoniacal and nitrate nitrogen and form amino acids. They also synthesize carbodyrates, which act as retarder of nitrogen loss. This effect is much more pronounced in light than in the dark indicating that the algal activity is greately enhanced by light.

An interesting observation is that chlorella, which does not play a significant role in fixing atmospheric nitrogen in the soil has been found to be the best checker of nitrogen loss amongst the three algae used in the present investigation. The important observation is that there is a positive correlation between the growth rate of algae, as indicated by the carbon saving, and their power of retarding the nitrogen loss. The order of carbon saving and so also of the power of retarding the nitrogen loss in the present investigation is chlorella> Tolypothrix> Anabaena.

The most important observation which must be mentioned here is that the power of algae to check nitrogen loss is much less than that of the organic material, wheat straw, undergrowing slow oxidation both in absence and presence of phosphates. The results recorded below in table No. 17 make this very clear.

TABLE 17

Decrease in Nitrogen loss (Percentage)

IN 240 DAYS

Nitrogen loss retarder	No. Phosphate	Tata Basic Slag
Chlorella	3·8	7·8
Tolypothrix	2·6	6·6
Anabaena	1·9	5·4
Wheat Straw	15·6	20·1

Another important observation is that the retarding effect of algae on nitrogen loss from ammonium sulphate is more pronounced in presence of Tata Basic Slag and wheat straw, indicating that these materials encourage the growth of algae. This can be seen very clearly from the results recorded in table No. 18.

TABLE 18

Decrease in Nitrogen lost in gm. by different algae in 240 days

(Difference over the respective controls)

Nitrogen loss retarder	No Phosphate	Tata Basic Slag
Chlorella alone ...	0·0138	0·0200
Chlorella with straw ...	0·0221	0·0240
Tolypothrix alone ...	0·0094	0 0156
Tolypothrix with straw ...	0·0138	0·0192
Anabaena alone ...	0·0069	0·0112
Anabaena with straw ...	0·0103	0·0137

Taking the case of chlorella the decrease in nitrogen loss is 0·0138 gm. in the absence of organic material and phosphate but in the presence of wheat straw and Tata basic slag, the decrease is 0·0240 gm., that is almost double. Similar is the case with the remaining two algae, Tolypothrix and Anabaena. De and Co-workers (3,4) while working with blue green algae, observed greater nitrogen fixation with crops than in their absence. This is due to the existence of more organic matter in the soil with crops. The fixation of nitrogen opposes nitrogen loss from soils.

Thus from these experimental observations it can be concluded that the three algae namely chlorella, Tolypothrix and Anabaena retard nitrogen loss from the soil—more in presence of organic material and phosphate than in their absence. The organic material, wheat-straw, specially when aided by phosphates, checks nitrogen loss much more than the loss checked by the algae.

REFERENCES

1. Bizell, J. A, *Cornell Agri. Exp. Sta. Mm.* 256, 1944.
2. Bracken, A. F. & Greaves, J. E., *Journ. Amer. Soc. Agron.* 29, 1130, 1929.
3. De P. K. & Mandal, L. N., *Soil. Sci.* 81 ; 453, 1956.
4. De P. K. & Sulaiman, M., *Ind. J. Agric. Sci* , 22 : 375, 1942.
5. Dhar, N. R., *Influence of light on some biochemical processes*, 1939.
6. Dhar, N. R., *J. Ind. Chem. Soc.*, 27. 425, 1950.
7. Dhar, N. R., *Kungl. Lanbrunkshogskolans Annaler Vol.* 21, 1954.
8. Dhar, N. R. & Ghildyal, B. N. P., *Proc. Nat. Acad. Sci. India* 20—1, 1957.
9. Dhar, N. R, & Gopala Rao, G., *J. Ind. Chem. Soc. Sir P. C. Ray Memorial volume pp.* 82, 1933.
10. Dhar, N. R. & Mukherji, S. K., *J. Ind. Chem. Soc.* 11 : 727, 1934.
11. Dhar, N. R. & Pant, N. N., *Nature*, 142 : 878, 1942.
12. Dhar, N. R., Tondon, S. P. & Mukherji, S. K., *J. Ind Chem. Soc.* 112, 67, 1935.
13. Fritsch, F. E. & De, P. K. *Nature*, 142 : 878, 1938.
14. Gainey, P. L., *Journ. Amer. Soc. Agron*, 639, 1929.
15. Gautier, A. & Drouin, R, *R. Compt. Rend Acad, Sci.*, 113 : 820-25, 1891,
16. Lohnis, F. & Fred, F. B., *Text-Book of Agricultural Bacteriology pp.* 224, 1923.
17. Lyon, T. L., *Proc First Inter. Congrl. Soil Sci* , 619-627, 1927.
18. Megitt, *Mem. Dept. Agri. Wd. Chem.*, 1923.
19. Snyder, H., *Minn. Agri. Exp. Sta, Bull.* 153, 1898.
20. Swanson, C. O. *Jansen, Agri., Exp* , *Sta. Bull.* 119, 1914.
21. Swanson, C. O. *Janson, Agri., Exp.*, *Sta. Bull.* 220, 1918.
22. Watanabe, A., *Bot. Mag. Tokyo*, 69 : 820-21, 1956.
23. Wilson, B. D., *Proc. Soil Sci., Amer. Vol. VI*, 196, 1911.

INFLUENCE OF LIGHT INTENSITY, ORGANIC MATTER AND PHOSPHATE ON : (A) NITROGEN FIXATION AND (B) AVAILABILITY OF P_2O_5 IN THE PRESENCE AND ABSENCE OF ANABAENA NAVICULOIDES AND CHLORELLA PYRENOIDOSA

By

N. R. DHAR *and* G. N. BHAT

Sheila Dhar Institute of Soil Science, University of Allahabad, Allahabad

ABSTRACT

When carbonaceous materials, mixed with sand, alone or reinforced with phosphates and inoculated with algae of two types, namely, Anabaena naviculoides and Chlorella pyrenoidosa, are allowed to undergo slow oxidation, there is a slight saving of carbon. It is observed that the process is mainly due to the small increase in the carbon content due to photosynthesis and its oxidation and consequent fixation of nitrogen.

INTRODUCTION

Attempts have been by a number of soil scientists to correlate the phenomenon of nitrogen fixation in rice fields, specially in India, to the activity of blue green algae. But, De and Dutta (1) failed to grow blue green algae and account for any nitrogen fixation with rice soils under laboratory conditions. Light is indispensable for the growth of algae. Allison and Hoover (2) have, however, shown that some species of blue green algae are also able to grow and fix nitrogen in the dark in presence of suitable, carbohydrates. According to these workers, growth and nitrogen fixation are increased by sugars even in light.

According to Pearsall (3) the blue green algae are frequently met in abundance in fresh water, specially in neutral and alkaline waters with a relatively high content of dissolved organic matter and a low content of nitrate. These observations show that blue green algae can grow better provided a sufficient amonnt of organic matter is already present in the soil.

In order to throw further light on this problem, an attempt has been made in this paper to obtain quantitative date on nitrogen fixation caused by the slow oxidation of carbonaceous materials like glucose and wheat straw mixed

with sand in the presence and absence of two types of algae, namely, Anabaena naviculoides and Chlorella pyrenoidosa.

To study the effect of phosphate, Tata basic slag has been utilised as a source of phosphate.

EXPERIMENTAL

The following media as recommended by De (4) were used for the growth of Anabaena naviculoides and Chlorella pyrenoidosa:-

Medium for Anabaena			Medium for Chlorella		
KNO_3	...	0.2 gm.	NH_4NO_3	...	0.2 gm.
K_2HPO_4	...	0.2 gm.	K_2HPO_4	...	0.2 gm.
$MgSO_4.7H_2O$	...	0.2 gm.	$MgSO_4.7H_2O$	...	0.2 gm.
$CaCl_2$	...	0.1 gm.	$CaCl_2$	...	0.1 gm.
$FeCl_3$(1%)	...	2 drops	$FeCl_3$(1%)	...	2 drops
Distilled H_2O	...	1000 cc.	Distilled water	...	1000 cc.

For experiments 100 gms. of air-dried sand were taken in clean, white enamelled dishes to which carbonaceous materials were added containing 0.8% organic carbon with and without phosphate sources to the extent of 0.5% P_2O_5. One set of experiments was exposed to light from a 100 Watt electric bulb and an identical set kept beside the exposed one covered with a thick black cloth in order to cut off light. The identical sets of experiments were started simultaneously and inoculated with Anabaena naviculoides or Chlorella pyrenoidosa, already growing in the above medium. Temperature was noted regularly.

After definite intervals of time, composite samples were taken out and analysed for organic carbon, total nitrogen, available nitrogen, available P_2O_5 and free amino acid content.

The amino acids identified have been abbreviated as follows:-

GIY	for	Glycine	Ar	for	Arginine
Al	,,	Alanine	Asp	,,	Aspartic acid
Va	,,	Valine	As	,,	Asparagine
Pro	,;	Proline	Glu	,,	Glutamic acid
His	,,	Histidine	Leu	,,	Leucine
Ly	,,	Lycine	Threo	,.	Threonine
			Se	,,	Serine

Estimation of carbon: The total organic carbonin a sımple of sand or other materials was estimated according to the method of Robinson, Mclean and Williams (5).

Estimation of nitrogen: This was estimated by Salicylic acid Reduction method (6).

Amino acids: Identification and separation of amino acids was undertaken with the help of Paper Chromatography.

Quantitative estimations were done colorimetrically.

Available P_5O_5: This was estimated by 1% Citric acid method.

Av. temp. 29°C

TABLE 1

100 gms/sand 0·8% C as glucose Anabaena naviculoides

Period of exposure in days	Total carbon %	Total nitrogen %	Efficiency	NH_3—N. (mgms) %	NO_3—N (mgms) %	Amino acide identified (Chromatographically).	Amount of amino acids with respect to glycine (mgms. %) Colorimetrically)	Total available P_2O_5 %
					LIGHT			
0	0·8940	0·0040	...	...	0·86	...	...	0·0042
30	0·6526	0·0108	44·9	0·4	0·9	Al, Va. Ly, Threo Pro, Gly.	0·4255	0·0068
60	0·5869	0·0138	45·1	0·8	1·3	Al. Va, Ly, Threo Pro, Glu.	0·5438	0·0075
90	0·5521	0·0154	45·2	1·1	1·6	Al, Va, Ly, Threo, Pro, Glu, Asp.	0·6165	0·0083
120	0·5249	0·0160	43·0	1·1	1·8	Al, Va, Ly, Threo, Pro, Glu. Asp.	0·5878	0·0089
					DARK			
0	0·8040	0·0040	...	...	0·86	...	...	0·0042
30	0·6701	0·0067	20·1	traces	0·9	Al, Va, Gly.	0·1666	0·0059
60	0·6082	0·0080	20·4	traces	1·1	Al, Va, Ly, Gly.	0·2215	0·0069
90	0·5770	0·0087	20·7	0·3	1·1	Ly, Al, Va, Glu, Threo.	0·2751	0·0075
120	0·5517	0·0090	19·8	0·4	1·2	Ly, Al, Va, Glu, Threo.	0·2649	0·0079

TABLE 2

100 gms. Jamuna sand 0·8 % C as glucose 0·5 % P_2O_5 as Tata basic slag Anab. naviculoides.

				LIGHT				
0	0·7558	0·0037	...	...	0·81	...	...	0·2355
30	0·5749	0·0134	53·6	1·0	1·2	Ly, Al, Va, Glu, Threo Asp, Ar.	0·7398	0·2668
60	0·5082	0·0170	53·7	1·3	2·2	Ly, Al, Va, Glu, Threo Asp, Ar, Pro.	1·0356	0·2651
90	0·4728	0·0189	53·7	1·6	2·5	Ly, Va, Glu, Threo, Asp, Ar, Pro, As, Leu.	1·2365	0·2712
120	0·4423	0·0199	51·9	1·7	2·9	Ly. Va, Glu, Threo, Asp, Ar, Pro, As, Leu.	1·2266	0·2735
				DARK				
0	0·7558	0·0537	...	...	0·81	...	...	0·2355
30	0·5843	0 0077	23·3	0·3	0·9	Gly, Al, Va, Ly, Giu.	0·3386	0·2465
60	0·5227	0·0096	25·5	0·6	1·2	Al, Va, Ly, Glu, Threo.	4·4715	0·2578
90	0·4905	0·0106	26·0	0·7	1·4	Al, Va, Ly, Glu, Asp, Threo Leu.	0·5479	0·2626
120	0.4627	0·0112	25·5	0.7	0·6	Al, Va, Ly, Glu, Asp, Threo, Leu.	0·5437	0·2647

Av. temp. 29°C.

TABLE 3

100 gms. Jamuna sand 0·8 % C as Wheat straw Anabaena naviculoides.

Period of exposure on days	Total carbon %	Total nitrogen %	Efficiency	NH_3N. (mgms) %	NO_3-N. (mgms) %	Amino acids identified (Chromatographically).	Amount of amino acids with respect to glycine. (mgms. %) Colorime-(trically)	Total availbale P_2O_5. %
					LIGHT			
0	0·8034	0·0166	...	...	0·86	...	...	0·0042
60	0·6423	0·0242	47·1	1·2	1·4	Al, Va, Ly, Glu, Asp, Threo	0·6115	0·0065
120	0·5859	0·0269	47·3	1·4	2·2	Al, Va, Ly, Glu, Asp, Threo, Leu, Pro.	1·0612	0·0076
180	0·5443	0·0283	45·1	1·6	2·6	Al, Va, Ly, Glu, Asp, Threo, Leu, Pro.	1·3107	0·0081
					DARK			
0	0·8034	0·0166	...	...	0·86	...	...	0·0042
60	0·6601	0·0196	20·9	0·7	1·0	Al, Va, Asp, Glu.	0·4168	0·0057
120	0·5889	0·0212	21·4	0·9	1·3	Al, Va, Asp, Glu, Ly, Threo.	0·6975	0·0065
180	0·5590	0·0216	20·4	1·1	1·7	Al, Va, Asp, Glu, Ly, Threo.	0·8586	0·0068

TABLE 4

100 gms. Jamuna sand 0·8 % C as Wheat straw 0·5 % P_2O_5 as Tata basic slag Anab. naviculoides.

				LIGHT				
0	0·7548	0·0156	...	...	0·81	...	...	0·2354
60	0·5620	0·0265	56.5	1·7	2·2	Al, Va, As, Threo Ly, Glu, Asp, Leu.	1·2535	0·2534
12.)	0·5011	0·0300	56·7	1·9	3·1	Al, Va, As, Threo, Ly, His, Ar, Glu, Asp, Pro.	1·8556	0·2606
180	0·4667	0·0315	55·1	2·0	3·7	Al, Va, As, Threo, Ly, His, Ar, Glu, Asp, Se.	2·2582	0·2654
				DARK				
0	0·7548	0·0156	...	...	0·81	...	...	0·2354
60	0·5706	0·0204	28·8	0·9	1·4	Al, Va, Glu, Ly, Asp, Threo.	0·7712	0·2459
120	0·5141	0·0221	27·0	1·0	2·1	Al, Va, Glu, Ly, Asp, Ar, His, Threo.	1·1635	0·2537
180	0·4811	0·0228	26·3	1·1	2·3	Al, Va, Glu, Ly, Asp, Ar, His, Threo.	1·5086	0·2568

Av. temp. 29°C.

TABLE 5

100 gms. Jamuna sand 0·8 % C as glucose Chlorella pyrenoidosa

Period of exposure in days	Total carbon %	Total nitrogen %	Efficiency	NH_3—N, (mgms) %	NO_3—N. (mgms) %	Amino acids indentified (Chromatographically)	Amino acids amount with respect to glycine, (mgms. %) Colorimetrically)	Total available P_2O_5.
					LIGHT			
0	0·8040	0·0040	...	o/o	0·86	—	...	0·0042
30	0·6509	0·0104	41·8	0·3	0·9	Al, Va, Ly, Gly,	0·3104	0·0063
60	0·5853	0·0132	42·0	0·6	1·2	Al, Va, Ly, Glu, Threo.	0·4253	0·0072
90	0·5506	0·0147	42·0	0·8	1·5	Al, Va, Ly, Glu, Threo Pro.	0·4916	0·0081
120	0·5231	0·0153	40·2	0·9	1·7	Al, Va, Ly, Glu, Threo Pro.	0·4734	0·0085
					DARK			
0	0·8040	0·0040	...	...	0·86	...	...	0·0042
30	0·6699	0·0066	19·3	traces	0·9	Al, Va, Gly.	0·1615	0·0057
60	0·6082	0·0080	20·4	traces	1·1	Al, Va, Gly, Ly.	0·2190	0·0068
90	0·5767	0·0087	20·6	0·3	1·1	Al, Va, Glu, Threo	0·2696	0·0075
120	0·5515	0·0089	19·4	0·3	1·2	Al, Va, Ly, Glu, Threo	0·2594	0·0079

TABLE 6

100 gms. Jamuna sand 0·8 % C as glucose 0·5 % P_2O_5 as Tata basic slag pyrenoidosa

LIGHT								
0	0·7558	0·0037	...	...	0·81	...	...	0·2355
30	0·5727	0·0129	50·2	0·8	1·1	Al, Va, Glu, Ly, Asp, Threo	0·6758	0·2561
60	0·5062	0·0163	50·4	1·0	2·1	Al, Va, Glu, Asp, Ar, Ly, Threo.	0·9382	0·2652
90	0·4707	0·0181	50·5	1·3	2·4	Ly, Va, Glu, Asp, Ar, Pro, Leu, Threo.	1·1015	0·2705
120	0·4409	0·0191	48·9	1·4	2·8	Ly, Va, Glu, Asp, Ar, Threo, Pro, Leu.	1·0955	0·2726
DARK								
0	0·7558	0·0037	...	...	0·81	---	...	0·2355
30	0·5840	0·0076	22·7	0·3	0·9	Al, Gly, Ly, Va, Glu.	0·3365	0·2461
60	0·5219	0·0096	25·2	0·6	1·2	Al, Ly, Va, Glu, Threo.	0·4693	0·2575
90	0·4901	0·0105	25·1	0·7	1·4	Al, Ly, Va, Glu, Threo, Asp, Leu.	0·5432	0·2626
120	0·4624	0·0111	25·2	0·7	1·6	Al, Ly, Va, Glu, Threo, Asp, Leu.	0·5418	0·2644

Av. temp. 29°C

TABLE 7

103 gms: Jumuna sand 0·8 % C as Wheat straw Chlorella pyrenoidosa

Period of cxpo-sure in days	Total carbon %	Total nitrogen %	Efficiency	NH_3—N, (mgms) %	NO_3—N. (mgms) %	Amino acids identified (Chromatographically)	Amount of amino acids with respect to glycine, (mgms. %) (Colorimetrically)	Total P_2O_5. %
					LIGHT			
0	0·8034	0·0166	...	...	0·86	...	...	0·0042
60	0·6413	0·0237	43·8	0·9	1·3	Al, Va, Ly, Asp, Glu.	0·5538	0·0061
120	0·5830	0·0263	44·0	1·0	2·1	Al, Va, Ly, Asp, Glu, Threo, Leu.	0·9365	0·0069
180	0·5433	0·0275	41·9	1·4	2·4	Al, Va, Ly, Asp, Glu, Threo, Leu.	1·2189	0·0078
					DARK			
0	0·8034	0·0166	...	...	0·86	...	...	0·0042
60	0·6596	0·0196	20·8	0·7	0·9	Al, Va, Glu, Asp.	0·3995	0·0056
120	0·5883	0·0211	20·9	0·8	1·4	Al, Va, Ly, Glu, Asp, Threo	0·6785	0·0063
180	0·5585	0·0215	20·0	0·9	1·8	Al, Va, Ly, Glu, Asp, Threo,	0·8522	0·0065

TABLE 8

100 gms. Jamuna sand 0·8 % C as Wheat straw 0·5 % P_2O_5 as Tata basic slag Chlorella pyrenoidosa

				LIGHT				
0	0·7548	0·0156	...	...	0·81	...	...	0·2354
60	0·5594	0·0260	53·2	1·3	2·1	Al, Va, Ly, Glu, As, Asp, Threo, Pro.	1·1965	0·2528
120	0·4997	0·0292	53·3	1·6	2·9	Va, Ly, Glu, Asp, Ar, Threo, His, Leu, As.	1·7562	0·2601
180	0·4647	0·0306	51·7	1·7	3·6	Va, Ly, Glu, Asp, Ar, Threo, His, Leu, As.	2·2176	0·2649
				DARK				
0	0·7548	0·0156	...	...	0·81	...	...	0·2354
60	0·5756	0·0203	26·2	0·8	1·4	Al, Va, Asp, Glu, Ly, Threo,	0·7695	0·2456
120	0·5138	0·0220	26·5	1·1	2·0	Al, Va, Asp, Ly, Glu, Ar, His, Threo.	1·1608	0·2535
180	0·4807	0·0227	25·9	1·1	2·4	Al, Va, Asp, Glu, Ly, Ar, His, Threo.	1·4995	0·2566

TABLE 9

100 gms. Jamuna sand 0·8 % C as glucose.

Av. Temp. 29°C.

Period of exposure in days	Total carbon %	Total nitrogen %	Efficiency	HN_3—N. (mgms) %	NO_3—N. (mgms) %	Amino acids identified (Chromatographically)	Amount of amino acids with respect to glycine (mgms. %) Colorimetrically).	Total P_2O_5. %
LIGHT								
0	0·8040	0·0040	...	...	0·86	...	...	0·0042
30	0·6342	0·0103	37·1	0·3	0·90	Gly, Al, Va, Ly.	0·2987	0·0061
60	0·5661	0·0131	38·2	0·7	1·50	Al, Va, Ly, Glu, Threo	0·4061	0·0072
90	0·5319	0·0145	38·5	0·9	1·90	Al. Va, Ly, Glu, Pro, Threo.	0·4785	0·0078
120	0·5084	0·0151	37·5	0·9	2·10	Al, Va, Ly, Glu, Threo, Pro.	0·4681	0·0082
DARK								
0	0·8040	0·0040	...	...	0·86	...	...	0·0042
30	0·6695	0·0075	18·5	traces	0·90	Gly, Al, Va.	0·1575	0·0057
60	0·6075	0·0079	19·8	traces	1·10	Al, Va, Ly, Gly.	0·2054	0·0066
90	0·5757	0·0086	20·1	0·3	1·10	Al, Va, Ly, Glu, Threo.	0·2665	0·0072
120	0·5509	0·0089	19·3	0·3	1·20	Al, Va, Ly, Glu, Threo.	0·2581	0·0076

TABLE 10

100 gms. Jamuna sand 0·8 % C as glucose 0·5 % P_2O_5 as Tata basic slag

				LIGHT				
0	0·7558	0·0037	...	...	0·81	...	...	0·2355
30	0·5547	0·0128	45·2	0·9	1·40	Al, Va, Ly, Glu, Threo, Asp.	0·6653	0·2556
60	0·4875	0·0162	46·5	1·1	2·50	Al, Va, Ly, Glu, Threo, Asp, Ar.	0·9235	0·2649
90	0·4534	0·0179	46·9	1·3	3·00	Va, Ly, Glu, Asp, Ar, Threo, Leu, Pro.	1·0735	0·2696
120	0·4267	0·0190	46·4	1·5	3·30	Va, Ly, Glu, Asp, Ar, Threo, Leu, Pro.	1·0702	0·2721
				DARK				
0	0·7558	0·0037	...	...	0·81	...	...	0·2355
30	0·5835	0·0076	22·6	0·3	0·90	Gly, Al Va, Ly, Glu.	0·3345	0·2461
60	0·5217	0·0095	24·7	0·6	1·20	Al, Va, Glu, Ly, Threa.	0·4659	0·2573
90	0·4896	0·0104	25·1	0·7	1·40	Al, Va, Glu, Ly, Asp, Threo, Leu.	0·5415	0·2621
120	0·4617	0·0110	24·8	0·7	1·60	Al, Va, Glu, Ly, Asp, Threo, Leu.	0·5398	0·2640

TABLE 11

100 gms. Jamuna sand 0·8 % C as Wheat straw

Av. temp. 29°C.

Period of exposure in days.	Total carbon. %	Total nitrogem %	Efficiency	$NH_3 - N$ (mgms) %	$NO_3 - N$. (mgms) %	Amino acids identified. (Chromatographically)	Amount of amino acids with respect to glycine. (mgms. %) (Colorimetrically).	Total
				LIGHT				
0	0·8034	0·0166	...	...	0·86	...	...	0·0042
60	0·6267	0·0236	39·6	1·0	1·60	Al, Va, Glu, Asp, Ly.	0·5196	0·0058
120	0·5665	0·0261	40·1	1·2	2·50	Al, Va, Glu, Asp, Ly, Threo, Leu.	0·9658	0·0067
180	0·5282	0·0274	39·2	1·5	2·90	Al, Va, Glu, Asp, Ly, Threo, Leu.	1·2065	0·0073
				DARK				
0	0·8034	0·0166	...	...	0·86	...	...	0·0042
60	0·6592	0·0195	20·1	0·7	0·90	Al, Glu, Va, Asp.	0·3905	0·0054
120	0·5879	0·0210	22·4	0·9	1·40	Al, Giu, Va, Asp, Ly, Threo	0·6728	0·0061
180	0·5580	0 0215	19·9	1·0	1·80	Al, Glu, Va, Asp, Ly, Threo.	0·8416	0·0065

TABLE 12

100 gms. Jamuna sand 0·8 % C as Wheat straw 0·5 % P_2O_5 as Tata basic slag.

LIGHT

0	0·7548	0·0156	...	...	0·81	...	...	0·2354
60	0·5450	0·0258	48·6	1·5	2·60	Al, Va, Ly, Asp, Glu, As, Threo Pro.	1·1352	0 2523
120	0·4823	0·0291	49·5	1·7	3·50	Ly, Va, Asp, Glu, As, Ar, His, Threo, Leu.	1·7169	0·2595
180	0·4517	0·0305	49·1	1·8	4·00	Ly, Va, Asp, Glu, As, Ar, His, Threo, Leu.	2·1965	0·2641

DARK

0	0·7548	0·0156	...	...	0·81	...	...	0·2354
60	0·5752	0·0202	25·6	0·9	1·50	Al, Va, Ly, Glu, Asp, Threo,	0·7475	0·2454
120	0·5135	0·0219	26·1	1·1	2·00	Al, Va, Ly, Glu, Asp, Ar, His, Threo.	1·1393	0·2531
180	0·4801	0·0227	25·8	1·2	2·40	Al, Va, Ly, Glu, Asp, Ar, His, Threo.	1·4755	0·2663

DISCUSSION

An examination of the experimental results (vide Tables 1 to 4) shows that when organic materials like glucose or wheat straw, mixed with sand and inoculated with Anabaena naviculoides, are allowed to undergo slow oxidation in air, there is a little saving of carbon and thereby a slight increase in the nitrogen fixed in comparison to the uninoculated systems (vide Tables 9 to 12). It is also observed that in the systems inoculated with Chlorella pyrenoidosa (vide Tables 5 to 8) the saving of carbon is less than the systems containing Anabaena naviculoides, showing thereby the activity of Anabaena naviculoides more pronounced than Chlorella pyrenoidosa in carrying out such processes. In the dark; the systems inoculated with either of the two types of algae show that the amount of carbon oxidised or nitrogen fixed is more or less the same as in the absence of such algae, indicating thereby that these algae grow only in the presence of light. Moreover, it seems that organic matter activates these organisms so that some carbon of the system is saved which leads to the fixation of atmospheric nitrogen. These observations are in agreement with the experimental results of De and Sulaiman (7) and of De and Mandal (8). According to these workers, nitrogen fixation was better in the presence of a crop than in its absence. They attributed this stimulating effect to the increased supply of CO_2 resulting from the respiration and decomposition of roots of higher plants.

Moreover, from the present experimental results it can be revealed that the efficiency, i. e. the amount of nitrogen fixed in mgms. per gram of carbon oxidised, is greater in light in the presence of algae than in their absence. This is due to the fact that these algae contribute some organic carbon and the increased efficiency is mianly due to the increase in corbon content due to photosynthesis and its oxidation and the consequent fixation of nitrogen.

Acccrding to Dhar (9) grasslands are more fertile, i.e. rich in total nitrogen and available phosphate. It has been assumed that grass grows in the presence of light and there is also a decay and slow oxidation of the dead parts of roots, stems etc. The grasslands, therefore. show a picture of carbohydrate photosynthesis and carbohydrate oxidation side by side, and, this process appears to be more or less allied to the growth of the algae and the nitrogen fixation in the systems containing organic matter. A part of organic matter of the systems undergoes slow oxidation and fixes more nitrogen in light than in the dark, and, a part of the organic matter lost might be compensated by the growth of the algae.

It can also be observed that the rate of efficiency of nitrogen fixation is greater in the systems inoculated with Anabaena naviculoides than in the

systems inoculated with Chlorella pyrenoidosa. The observations show that Anabaena naviculoides carries out dual functions of simultaneous growth and nitrogen fixation with the result that the efficiency of nitrogen fixation is more pronounced in the presence of this algae than in that of Chlorella pyrenoidosa.

Furthermore, it is clear from these experimental data that the amount of corbon oxidised, nitrogen fixed and the efficiency of nitrogen fixation are greatly enhanced when Tata basic slag as phosphate source is added to the systems. It is observed that the saving of carbon or the efficiency of nitrogen fixation is more pronounced in the phosphated systems containing organic matter without algae. These observations show that the algae thrive better in the presence of phosphates than in their absence. According to De and Sulaiman (7) and Okuda and Yamaguchi (10) phosphate and calcium supplies appear to be the important limiting factors for the growth of blue green algae in certain rice fields.

It is also observed that the increase in the availability of phosphate in the algae inoculated systems is slightly greater than in the systems without algae. These observations can be explained on the basis that besides the slow action of carbonic acid or organic acids, in increasing the availability of phosphate, the organic phosphorus of nucleic acids, nucleotides etc. of the alhal material is made available by dephosphorylation. Bower (11) presumed that mononucleotides, nucleic acids and nucleoproteins, introduced into the soil by the incorporation of plant and animal residues or sysnthesised by soil micro-organisms, contain a major portion of the soil organic phosphorus.

From a perusal of the foregoing experimental results it can be concluded that the systems containing organic matter mixed with sand and inoculated with Anabaena naviculoides in the presence or absence of phosphates, show a slight increase in the ammoniacal nitrogen and a slight decrease in the nitric nitrogen as compared to the analogous uninoculated systems. On the other hand, in the systems inoculated with Chlorella pyrenoidosa, there is a slight decrease in both ammoniacal as well as nitric nitrogen in contrast to the uninoculated systems. These algae absorb nitrogen mostly in the nitrate form. Magee and Burris (12), using Nostoc muscovum supplied with an adequate quantity of molybdenum, found assimilation to be rapid when the source was nitrate as compared with elementary nitrogen.

A further examination of the experimental results reveals that the systems inoculated with Anabaena naviculoides record greater yields of amino acids as compared to the uninoculated systems. The amino acid chromatograms were

distinct and in addition to the amino acids detected in the uninoculated systems, serine was frequently detected in the presence of Anabaena naviculoides. In the systems inoculated with Chlorella pyrenoidosa, the amount as well as the number of amino acids did not show any significant change in comparison to the uninoculated systems. The decomposition and oxidation of algae bodies may be responsible for the increased amount of or the number of amino acids.

From an overall look at the experimental results, it appears that the fixation of nitrogen depends chiefly on the amount of carbonaceous compounds oxidised and aided by the light intensity and phosphates.

REFERENCES

1. De, P. K. & Dutta, N. R., *Ind. J. Agric. Sci.*, 22, 375 (1952).
2. Allison, F. E & Hoover, S. R., *Trans. 3rd. Intern. Congr. Soil Sci., Oxford, Comm.* 145 1935).
3. Pearsall, W. H., *J. Ecol.*, 20, 241 (1932).
4. De, P. K., *Proc. Roy. Soc., London, B.*, 127, 121 (1939).
5. Robinson, Mclean & Williams, *J. Agri. Sci.*, 19, 315 (1929).
6. Treadwell, F. P. & Hall, W. T., *Analytical Chemistry*, Vol. II, p. 493-494 (1947).
7. De, P. K. & Sulaiman, M., *Ind. J. Agric. Sci.*, 20, 327 (1950).
8. De, P. K. & Mandal, L. N., *Soil Science*, 81, 453 (1956).
9. Dhar, N. R., *Presi. Addr. 48th. Indian Sci. Congr.* (1961).
10. Okuda, A. and Yamaguchi, M., *Mem. Res. Inst. Food Sci.*, Kyoto Univ., 4, 1 (1952).
11. Bower, C. A., *Iowa Agric.* Exp. Sta. Res. Bull. 362 (1949).
12. Magee, W. E. and Burris, R. H., *Amer. J Bot*, 41, 777 (1954).

COMPOSTING OF WATER HYACINTH IN PRESENCE AND ABSENCE OF ALAGE

By

N. R. DHAR *and* B. S. GUPTA

Dhar Institute of Soil Science, University of Allahabad

ABSTRACT

Water hyacinth (Eichornia crassipes) grows everywhere in great abundance and frequently its eradication is a big problem.

Experiments have been carried on with water hyacinth for its composting and obtaining available nitrogen, phosphate and potash from the compost formed by mixing it with different basic slag and the three algae, Chlorella, Anabaena and Tolypothrix.

Experimental results show that these algae, specially Anabaena and Tolypothrix improve the quality of the compost from the nitrogen viewpoint.

Phosphates markedly improve the nitrogen content when the water hyacinth compost is obtained by mixing it with basic slags.

The trace elements present in the basic slags markedly improve the quality and the productive power of the water hyacinth compost.

Blue green algae was first obtained by Frank in 1889 and Drewes reported in 1928 that these algae can fix nitrogen from air. Later on Drewes's idea was supported by Allison and Morris, Skoog, Bortels and Fogg. Subsequently, Fritsch classified these algae which was followed by on further modification of Williams and Burris, Fogg, Watanabe and Henriksson. Allen, Tamiya, Watanabe, Allen and Aron reported that anabeana and tolypothrix are nitrogen fixer but chlorella does not, but all these three algae play an important part in the improvement of land fertility nd plant growth. These algae affect plant growth in different manners. They may add some organic matter to the soil, help bind the soil particles of swamp soil and fix atmospheric nitrogen. Recently Engle and Mc-Murtrey have shown that adding green algae to water culture in which tobacco is growing improves

the aeration of solution, if the culture is kept in light, resulting not only an improvement growth of the tobacco plants, but also in an increased immunity of their roots fungal infections. De and Sulaiman, Singh have reported the residual effect of nitrogen fixed by these algae.

On the other hand Dhar and others have been advocating the application of organic substances for increasing land fertility by direct incorporation of organic matter or composting with basic slag which is a buy product of steel industries. This compost is not only rich in nitrogen, phosphate, lime, potash but also the several trace elements which helps in plant growth. Dhar has further emphasized that greater amounts of nitrogen are fix in light then in dark and this is universally applicable when organic matter is incorporated with soil.

Water hyacinth, which is rich in potash and nitrogen, has been utilized by us for preparing the compost with different basic slag. The purpose of the present investigation was to determine the relative efficiency in nitrogen fixation by different algae (i. e. Chlorella, Anabeana and Tolypothrix) inocculated in the waterhyacith with or without basic slag.

EXPERIMENTAL

100 gms. of water hyacinth dried at 105° C for 5 to 6 hours and passed through a 30 mesh sieve, were taken in enamelled dishes 24 c. m. diameter. The different basic slags were added at the rate of 50% organic matter by weight (i. e.) 50gms in each plats). Basic Slags was previously well powered and passed through 100mesh sieve. The moisture level was maintained at 50% by weighting and adding water at intervals. Anabeana, Tolypothrix or Chlorella was added and the system were stirred with a glass rod daily in the first week and then alternate day, to facilitate the aeration. One set was exposed to the light of a 100 watt electric bulb with a reflector, hung at a vertical distance of about one and half feet above the table on which these dishes were placed. Another set of dishes containing in every detail the same materials were kept covered with a thick piece of black cloth in such a way that no light passes on plates, but air can contact with materials.

After 30 days, a known quantity of representative samples were taken out from the dishes and were dried and then estimated total carbon, total nitrogen, available nitrogen, available phosphates and pH of the mixture (sample s).

Analysis of Water hyacinth (Eichornia Crassipes)

Loss on ignition	... 63·238%	K_2O	... 4·8136%		
Ash	... 36·762%	P_2O_5	... 0·5580%		
Fe_2O_3	... 1·2396%				
Al_2O_3	... 0·0896%	P_2O_5 (A)	... 0·3876%		
CaO	... 2·2530%	Total Carbon	32·438%	C/N ratio	21·51
MgO	... 0·7760%	Total Nitrogen	1·5066%		

Analysis of Basic Slags

	Tata Basic Slag.	Durgapur Basic Slag	Kulti Basic Slag.	Rourkela Basic Slag.	German Basic Slag.	Belgian Basic Slag.
SiO_2	22·4600	18·8960	20·1640	19·9670	14·0466	15·2346
Fe_2O_3	15·6740	17·2880	16·4060	17·7660	15·8760	16·8474
Al_2O_3	5·4326	6·8744	6·4866	6·3666	3·0668	2·9354
CaO	38·8857	40·8886	41·1864	39·9943	42·3464	41·6868
MnO_2	2·8687	4·6634	3·0886	3·1675	4·8734	4·1864
MgO	4·1345	5·6723	4·8486	6·0789	4·9684	4·6785
K_2O	0·6458	0·3364	0·5460	0·6847	0·1842	0·2332
V_2O_5	0·4881	0·2876	0·3144	0·4088	0·6438	0·5488
Cr_2O_3	0·3897	0·2366	0·4088	0·3172	0·5678	0·4773
TiO_2	0·3126	0·2084	0·2743	0·3674	0·5784	0·4970
CuO	0·0048	0·0094	0·0136	0·0064	0·0062	0·0078
ZnO	0·0064	0·0176	0·0218	0·0076	0·0087	0·0066
P_2O_5 Total	17·7380	3·4868	4·1680	2·0684	17·8683	16·6640
P_2O_5 (A) 1 % citric Acid Soluble)	4·1020	1·4802	2·0340	0·8644	7·9672	7·6360

TABLE 1

Water hyacinth only

Period in Days	Total Carbon in gm.	Total Nitrogen in gms.	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	—	...	..	21·51	...	0·3875	...
30	26·0000	1·7127	0·04294	0·05984	0·10278	15·20	32·00	0·4851	6·25
60	22 4860	1·8200	0·06636	0·08872	0·15508	12·35	31·50	0·5597	6·15
90	20·0234	1·8931	0·07984	0·10246	0·18230	10·59	31·10	0·6196	6·05
120	18·1655	1·9348	0·07936	0·10206	0·18142	9·38	30·00	0·6800	6·10
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	0·3875	...
30	26·9364	1·6171	0·03846	0·05693	0·09539	16·63	20·00	0·4656	6·35
60	23·5000	1·6846	0·04644	0·07449	0·12093	13·97	19·88	0·5336	6·20
90	21·2366	1·7265	0 05123	0·08638	0·13761	12·32	19·84	0·5974	6·10
120	19·6733	1·7492	0·05466	0·09145	0·14611	11·24	19·00	0·6443	6·05

TABLE 2

Water hyacinth and Anabeana

LIGHT									
0	32·4384	1·5066	—	—	—	21·51	...	0·3875	—
30	26·0136	1·7187	0·04163	0·05966	0·10129	15·16	33·01	0·4826	6·25
60	22·5009	1·8235	0·06236	0·08694	0·14930	12·33	32·94	0·5555	6·15
90	20·0567	1·9028	0·07268	0·00696	0·16964	10·54	32·00	0·6139	6·00
120	18·2000	1·9522	0·07566	0·10236	0·17802	9·32	31·53	0·6733	6·00
DARK									
0	32·4384	1·5066	—	—	—	21·51	—	0·3875	—
30	26·9400	1·6173	0·03850	0·05700	0·09550	16·64	20·00	0·4658	6·35
60	23·5000	1·6850	0·04655	0 07444	0·12099	14·00	19·89	0·5346	6·20
90	21·2404	1·7259	0·05118	0·03640	0·13758	12·33	19·76	0·5966	6·10
120	19·6699	1·7500	0·05472	0·09140	0·14612	11·24	19·00	0·6429	6·05

TABLE 3

Water hyacinth and Tolypothrix

Period in Days	Total Carbon in gm.	Total Nitrogen in gms.	NH_3—N in gm.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	0·3375	...
30	26·0140	1·7185	0·04170	0·05970	0·10140	15·17	33·00	0·4838	6·30
60	22·4997	1·8240	0·06228	0·08700	0·14928	12·33	32·93	0·5564	6·15
90	20·0570	1·9024	0·07270	0·09712	0·16982	10·55	32·00	0·614	6·10
120	18·1986	1·9518	0·07570	0·10197	0·17767	9·33	31·49	0·6756	6·00
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	0·3875	...
30	26·9383	1·6177	0·03856	0·05689	0·09545	16·64	20·02	0·4663	6·40
60	23·5026	1·6874	0·04660	0·07453	0·12113	13·93	20·00	0 5344	6·25
90	21·2700	1·7273	0·05118	0·08646	0·13764	12·31	19·80	0·5968	6·10
120	19·6800	1·7508	0·05466	0·09096	0·14562	11·23	19·03	0·6450	6·05

TABLE 4

Water hyacinth and Chlorella

				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	0·3875	...
30	26·0148	1·7130	0·04142	0·05886	0·10028	15·19	32·13	0·4848	6·25
60	22·5078	1·8200	0·06167	0·08666	0·14833	12·36	31·56	0·5600	6·15
90	20·0688	1·8897	0·07226	0·09633	0·16859	10·56	31·00	0·6174	6·00
120	18·2186	1·9356	0·07526	0·10206	0·17732	9·42	30·33	0·6755	6·05
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	0·3875	—
30	26·9406	1·6177	0·03854	0·05693	0·09547	16·64	20·00	0·4663	6·35
60	23·5036	1·6850	0·04644	0·07399	0·12043	13·95	19·97	0·5336	6·20
90	21·2604	1·7265	0·05130	0·08650	0·13780	12·43	19·86	0·5986	6·10
120	19·6800	1·7500	0·05472	0·09146	0·14618	11·23	19·12	0·6439	6·00

TABLE 5

Water hyacinth and Rourkela Basic Slag

Period in Days	Total Carbon in gm.	Total Nitrogen in gms.	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of tha Mix.
				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	0·8197	...
30	24·9786	1·8798	0·05274	0·07456	0·12730	13·28	50·03	1·0688	7·85
60	21·0967	2·0624	0·08363	0·10378	0·18741	10·24	49·12	1·2674	7·65
90	18·5000	2·2024	0·09674	0·13064	0·22738	8·40	49·00	1·4386	7·50
120	16·9433	2·2503	0·09538	0·13058	0·22596	7·53	48·04	1·5656	7·60
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	0·8187	...
30	25·8800	1·7034	0·04386	0·06000	0·10386	15·22	30·00	1·0288	7·95
60	22·2684	1·8066	0 05516	0·07638	0·13154	12·38	29·50	1·2000	7·80
90	19·8774	1·8700	0·06700	0·09983	0·16688	10·68	28·94	1·3587	7·70
120	18·4000	1·8998	0·07588	0·11234	0·18822	9·70	28·16	1·4513	7·65

TABLE 6

Water hyacinth, Rourkela Basic Slag & Anabeana

				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	0·8197	...
30	24·9898	1·8940	0·05132	0·07438	0·12570	13·15	52·00	1·0674	7·85
60	21·1170	2·0896	0·07988	0·10233	0·18221	10·10	52·50	1·2624	7·60
90	18·5226	2·2155	0·09233	0·12768	0·22001	8·39	51·00	1·4340	7·50
120	16·9676	2·2637	0·09200	0·12756	0·21956	7·50	49·13	1·5600	7·65
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	0·8197	...
30	25·9000	1·7029	0·04386	0·06014	0·10400	15·22	30·00	1·0300	7·95
60	22·2700	1·8070	0·05500	0·07644	0·13144	12·37	29·50	1·2014	7·75
90	19·8768	1·8700	0·06714	0·09974	0·16688	10·67	28·94	1·3600	7·65
120	18·4012	1·9000	0·07569	0·11246	0·18815	9·70	28·15	1·4504	7·60

TABLE 7

Water hyacinth, Rourkela Basic Slag & Tolypothrix

Period in Days.	Total Carbon in gms.	Total Nitrogen in gms.	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	0·8197	...
30	24·9906	1·8951	0·05136	0·07432	0·12568	13·16	52·00	1·0682	7·85
60	21·1204	2·0906	0·07979	0·10240	0·18219	10·10	51·60	1·2634	7·60
90	18·5288	2·2160	0·09240	0 12774	0·22014	8·40	51·03	1·4356	7·55
120	16·9729	2·2640	0·09200	0·12748	0·21948	7·49	49·33	1·5624	7·65
				DARK					
0	32·4384	1·5066	...	...	—	21·51	...	0·8197	—
30	25·8846	1·7040	0·04379	0·06000	0·10379	15·23	29·98	1·0294	7·95
60	22·2700	1·8060	0·05498	0·07650	0·13148	12·40	29·47	1·2000	7·80
90	19·8790	1·8724	0·06678	0·09974	0·16652	10·68	28·96	1·3594	7·75
120	18·3936	1·8989	0·07600	0·11244	0·18844	9·70	28·17	1·4528	7·70

TABLE 8

Water hyacinth, Rourkela Basic Slag & Chlorella

				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	0·8197	...
30	24·9934	1·8800	0·05140	0·07500	0·12640	13·29	50·24	1·0674	7·80
60	21·1320	2·0630	0·07986	0·10304	0·18290	10·24	49·47	1·2666	7·65
90	18·5800	2·2036	0·08238	0·12884	0·22122	8·45	50·33	1·4363	7·50
120	17·0600	2·2500	0·09249	0·12900	0·22149	7·59	49·00	1·5634	7·65
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	0·8197	...
30	25·8900	1·7050	0·04400	0·05976	0·10376	15·18	30·12	1·0298	7·95
60	19·8760	1·8058	0·05530	0·07644	0·13174	12·38	29·51	1·2034	7·75
90	19·8766	1·8718	0·06688	0·10000	0·16688	10·66	28·96	1·3563	7·65
120	18·3894	1·9000	0·07579	0·11226	0·18805	9·68	28·15	1·4489	7·70

TABLE 9

Water hyacinth and Durgapur Basic Slag

Period in Days	Total Carbon in gms.	Total Nitrogen in gms.	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	1·1276	...
30	24·6134	1·9594	0·05333	0·03134	0·13467	12·55	58·00	1·4978	7·60
60	20·7336	2·1536	0·09000	0·13133	0·22133	9·63	56·00	1·7988	7·45
90	18·2144	2·3381	0·10678	0·14266	0·24944	7·78	56·78	2·0166	7·30
120	16·6000	2·3036	0·09943	0·13810	0·23783	7·20	...	2·2178	7·50
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	1·1276	...
30	25·7884	1·7504	0·04600	0·05748	0·11348	14·73	36·66	1·4206	7·70
60	22·1063	1·8836	0·06244	0·08739	0·14983	11·73	36·48	1·6867	7·60
90	19·6784	1·9660	0·07384	0·09999	0·17383	10·00	36·01	1·8638	7·45
120	18·2538	2·0100	0·07967	0·11304	0·19271	9·08	35·52	2·0237	7·40

TABLE 10

Water hyacinth, Durgapur Basic Slag & Anabeana

LIGHT									
0	32·4384	1·5066	...	...	...	21·51	...	1·1276	...
30	24·6440	1·9824	0·05168	0·08024	0·13192	12·44	61·01	1·4977	7·55
60	20·7946	2·2052	0·08746	0·12894	0·21640	9·50	60·12	1·7963	7·40
90	18·3000	2·3408	0·10184	0·14000	0·24184	7·82	59·04	2·0133	7·30
120	16·7064	2·3124	0·09826	0·13749	0·23575	7·23	...	2·2126	7·45
DARK									
0	32·4384	1·5066	...	...	...	221·51	...	1·1276	...
30	25·8000	1·7500	0·04568	0·06752	0·11320	14·75	36·69	1·4188	7·70
60	22·1009	1·8840	0·06236	0·08744	0·14980	11·70	36·46	1·6900	7·60
90	19·6724	1·9647	0·07400	0·09976	0·17376	10·06	36·00	1·8644	7·50
120	18·2604	2·0086	0·07973	0·11234	0·19257	9·09	35·50	2·0246	7·45

TABLE 11

Water hyacinth, Durgapur Basic Slag & Tolypothrix

Period in Days	Total Carbon in gms.	Total Nitrogen in gms.	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	1·1276	...
30	24·6524	1·9816	0·05174	0·08134	0·13308	12·47	61·12	1·4964	7·45
60	20·8034	2·2060	0 08754	0·12963	0·21717	9·48	60·24	1·7970	7·30
90	18·3466	2·3394	0·10206	0·14034	0·24240	7·84	59·16	2·0142	7·25
120	16·7506	2·3200	0·09848	0·13850	0·23698	7·22	...	2·2119	7·40
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	1·1276	...
30	25·7946	1·7500	0·04582	0·06744	0·11326	14·76	36·68	1·4200	7·70
60	22·1094	1·8844	0·06239	0·08744	0·14983	11·70	36·48	1·6900	7·55
90	19·6900	1·9660	0·07426	0·09964	0·17390	10·00	36·14	1·8628	7·40
120	18·2632	2·0106	0·07896	0·11268	0·19164	9·13	35·53	2·0226	7·35

TABLE 12

Water hyacinth, Durgapur Basic Slag & Chlorella

				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	..	1·1276	...
30	24·6544	1·9603	0·05204	0·08068	0·13272	12·58	58·36	1·4958	7·45
60	20·8126	2·1544	0·08826	0·13000	0·21826	9·69	58·00	1·7944	7·30
90	18·3499	2·3376	0·10334	0·14068	0·24402	7·84	59·00	2·0083	7·25
120	16·7626	2·3072	0·09895	0·13333	0·23228	7·26	...	2·2133	7·35
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	1·1276	...
30	26·7866	1·7513	0·04600	0·06752	0·11352	14·76	37·72	1·4200	7·70
60	22·1000	1·8844	0·06199	0·08744	0·14943	11·73	36·45	1·6889	7·55
90	19·6684	1·9653	0·07400	0·10000	0·17400	10·00	36·00	1·8540	7·45
120	18·2500	2·0067	0·07936	0·11284	0·19220	9·10	35·48	2·0219	7·40

TABLE 13

Water hyacinth and Kulti Basic Slag

Period in Days	Total Carbon in gms.	Total Nitrogen in gms.	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	1·4045	...
30	24·5896	1·9776	0·05404	0·08206	0·13610	12·44	60·00	1·8524	7·80
60	20·6674	2·2010	0·09567	0·14404	0·23971	9·39	59·00	2·1966	7·60
90	18·0000	2·3587	0·10946	0·16000	0·26946	7·59	59·02	2·5678	7·45
120	16·3674	2·3574	0·10672	0·15333	0·26005	6·95	...	2·8018	7·60
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	1·4045	...
30	25·7136	1·7567	0·04664	0·06814	0·11478	14·63	37·16	1·7709	7·95
60	22·0000	1·8992	0·06313	0·08844	0·15157	11·58	37·66	2 0724	7·80
90	19·5684	1·9875	0·07443	0·10673	0·18116	9·80	37·37	2·3268	7·65
120	18·1346	2·0360	0·08014	0·11500	0·19514	8·91	37·00	2·5763	7·55

TABLE 14

Water hyacinth, Kulti Basic Slag & Anabeana

				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	1·4045	...
30	24·6312	1·9906	0·04936	0·08113	0·13099	12·37	62·00	1·8466	7·75
60	20·7284	2·2209	0·09074	0·13994	0·23068	9·33	61·01	2·1834	7·55
90	18·1167	2·3762	0·10386	0·15664	0·26050	7·61	60·88	2·5544	7·40
120	16·5000	2·3604	0·10200	0·15066	0·25266	7·00	...	2·7866	7·50
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	1·4045	...
30	25·7200	1·7570	0·04684	0·06788	0·11472	14·64	27·17	1·7688	7·95
60	22·0123	1·9000	0·06288	0·08799	0·14087	11·60	37·68	2·0800	7·80
90	19·5700	1·9867	0·07500	0·10588	0·18088	9·84	37·37	2·3300	7·65
120	18·1334	2·0344	0·08000	0·11488	0·19488	8·92	37·00	2·5800	7·55

TABLE 15

Water hyacinth, Kulti Basic Slag & Tolypothrix

Period in Day	Total Carbon in gms.	Total Nitrogen in gms.	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2G_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	1·4045	...
30	24·6446	1·9902	0·01980	0·08200	0·13180	12·35	62·11	1·8444	7·65
60	20·7356	2·2268	0·09123	0·13886	0·23009	9·31	61·55	2·1700	7·50
90	18·1368	2·3789	0·10406	0·15648	0·26054	7·62	61·00	2·5600	7·40
120	16·5444	2·3666	0·10228	0·15090	0·25318	6·99	...	2·7900	7·50
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	1·4045	...
30	25·7166	1·7570	0·04700	0·06814	0·11514	14·62	37·18	1·7700	8·00
60	22·0058	1·8984	0·06350	0·08800	0·15150	11·59	37·65	2·0688	7·80
90	19·5700	1·9900	0·07466	0·10700	0·18166	9·79	37·40	2·3300	7·65
120	18·1300	2·0333	0·08000	0·11488	0·19488	8·90	37·00	2·5689	7·60

TABLE 16

Water hyacinth, Kulti Basic Slag & Chlorella

				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	1·4045	...
30	24·6480	1·9780	0·05000	0·07998	0·12998	12·46	60·44	1·8484	7·70
60	20·7400	2·2034	0·09206	0·13900	0·23106	9·42	60·00	2·1896	7·55
90	18·1424	2·3579	0·10388	0·15650	0·26038	7·70	60·00	2·5568	7·40
120	16·5504	2·35[illegible]9	0·[illegible]0236	0·15400	0·25636	7·02	...	2·7890	7·50
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	1·4045	...
30	25·7200	1·7570	0·04684	0·06830	0·11514	14·64	37·17	1·7700	7·95
60	22·0123	1·9000	0·06333	0·08850	0·15183	11·60	37·68	2·0700	7·75
90	19·5684	1·9879	0·07464	0·10688	0·18152	9·83	37·34	2·3284	7·60
120	18·1400	2·0352	0·08000	0·11500	0·19500	8·94	37·00	2·5708	7·55

TABLE 17

Water hyacinth and Tata Basic Slag

Period in Days.	Total Carbon in gms.	Total Nitrogen in gms.	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	—	—	—	21·51	...	2·4385	...
30	24·3964	2·0488	0·05560	0·08413	0·13963	11·94	66·55	3·2444	7·70
60	20·4384	2·2922	0·09783	0·14666	0·24449	8·96	65·46	3·8748	7·55
90	17·7684	2·4600	0·11230	0·16368	0·27589	7·22	64·93	4·4856	7·50
120	16·0360	2·4236	0·11064	0·16256	0·27320	6·62	.	4·9747	7·65
				DARK					
0	32·4384	1·5066	—	—	—	21·51	—	2·4385	...
30	25·6000	1·7802	0·04713	0·07066	0·11778	14·38	40·13	3·0986	7·75
60	21·7964	1·9333	0·06440	0·08978	0·15418	11·30	39·96	3·6316	7·65
90	19·3400	2·0169	0·07600	0·10799	0·18399	9·61	39·00	4·0888	7·55
120	17·9000	2·0666	0·08284	0·12064	0·20348	8·67	38·46	4·4804	7·50

TABLE 18

Water hyacinth, Tata Basic Slag & Anabeana

LIGHT									
0	32·4884	1·5066	—	—	—	21·51	...	2·4385	—
30	24·4384	2·0468	0·05500	0·08388	0·13888	11·93	67·55	3·2500	7·65
60	20·5000	2·3000	0·09654	0·14538	0·24192	8·91	66·50	3·8750	7·50
90	17·8622	2·4750	0·11068	0·16170	0·27238	7·22	66·44	4·4796	7·45
120	16·1384	2·4400	0·10660	0·16000	0·26660	6·62	...	4·9688	7·55
DARK									
0	32·4384	1·5066	—	—	—	21·51	...	2·4385	—
30	25·6000	1·7813	0·04689	0·07072	0·11761	14·37	40·14	3·0956	7·80
60	21·7964	1·9318	0·06396	0·88898	0·15294	11·28	39·97	3·6337	7·65
90	19·3384	2·0175	0·07566	0·10846	0·18412	9·58	39·00	4·0912	7·55
120	17·8954	2·0657	0·08246	0·12100	0·20346	8·66	38·45	4·4836	7·50

TABLE 19

Water hyacinth. Tata Basic Slag & Tolypothrix

Period in Days	Total Carbon in gms.	Total Nitrogen in gms.	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	...	...	—	21·51	...	2·4385	...
30	24·4412	2·0506	0·5486	0·08388	0·13874	11·93	68·14	3·2392	7·65
60	20·5223	2·3158	0·09578	0·14544	0·24122	8·86	68·00	3·8694	7·50
90	17·8884	2·4960	0·11046	0·16126	0·27172	7·18	68·00	4·4800	7·45
120	16·1684	2·4550	1·10800	0·16000	0·26800	6·59	...	4·9667	7·60
				DARK					
0	32·4384	1·5066	...	...	—	21·51	—	2·4385	...
30	25·6067	1·7802	0·04724	0·07058	0·11782	11·40	40·11	3·0969	7·80
60	21·8123	1·9294	0·06456	0·08956	0·15412	11·33	39·97	3·6400	7·65
90	19·3336	2·0186	0·07600	0·10794	0·18394	9·56	39·00	4·1034	7·50
120	17·9024	2·0648	0·08300	0·12066	0·20366	8·68	38·47	4·4786	7·46

TABLE 20

Water hyacinath, Tata Basic Slag & Chlorella

				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	2·4385	...
30	24·4444	2·0430	0·05466	0·08400	0·13866	11·96	67·05	3·2400	7·65
60	20·5444	2·2899	0·09566	0·14484	0·24050	9·00	66·94	3·8700	7·50
90	17·9100	2·4626	0·11000	0·16088	0·27088	7·27	66·00	4·4788	7·40
120	16·1914	2·4244	0·10800	0·15986	0·26786	6·67	...	4·9596	7·55
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	2·4385	...
30	25·6000	1·7866	0·04724	0·07060	0·11784	14·38	40·13	3·1000	7·75
60	21·8200	1·9300	0·06470	0·09000	0·15470	11·33	39·97	3·6333	7·60
90	19·3400	2·0175	0·07584	0·10844	0·18428	9·58	39·00	4·0798	7·50
120	17·8966	2·0666	0·08260	0·11967	0·20227	8·66	38·45	4·4688	7·45

TABLE 21

Water hyacinth and German Basic Slag

Period in Days	Total Carbon in gms.	Total Nitrogen in gms	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	4.3711	...
30	24·2404	2·1051	0·06034	0·09436	0·15470	11·54	73·13	6·2688	7·95
60	20·1384	2·4175	0·10536	0·14127	0·24663	8·33	74·06	7·5846	7·75
90	17·4506	2·5866	0·12467	0·15886	0·28353	6·74	72·12	8·7645	7·60
120	15·7283	2·5814	0·11986	0·15746	0·27732	6·09	...	9·8967	7·80
				DARK					
0	32·4384	1·5068	...	...	...	21·51	...	4·3711	...
30	25·4664	1·8205	0·04888	0·07445	0·12333	13·97	45·03	5·9847	8·05
60	21·6074	1·9898	0·06884	0·09346	0·16230	10·85	44·59	7·0314	7·85
90	19·1240	2·0945	0·07989	0·11327	0·19316	9·10	44·21	8·0066	7·70
120	17·6378	2·1617	0·08689	0·12688	0·21377	8·16	43·38	8·6749	7·65

TABLE 22

Water hyacinth, German Basic Slag & Anabeana

				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	4·3711	...
0	24·2826	2·1217	0·05698	0·09336	0 15034	11·44	75·41	6·2607	7·95
60	20·2104	2·4569	0·09674	0·13497	0·23171	8·32	77·73	7·5722	7·70
90	17·5766	2·6256	0·11836	0·15069	0·26905	6·69	76·00	8·7466	7·55
120	15·8667	2·5821	0·11226	0·14439	0·25665	6·11	...	9·8838	7·70
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	4·3711	..
30	25 4688	1·8207	0·04896	0·07439	0·12335	13·98	45·00	5·9836	8·10
60	21·6069	1·9900	0·06900	0·09350	0·16250	10·85	44·61	7·0289	7·85
90	19·1226	2·0936	0·07926	0·11378	0·19304	9·15	44·16	8·0076	7·65
120	17·6400	2·1624	0·08704	0·12666	0·21370	8·14	43·40	8·6764	7·65

TABLE 22

Water hyacinth, German Basic Slag & Anabeana

				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	4·3711	...
0	24·2826	2·1217	0·05698	0·09336	0 15034	11·44	75·41	6·2607	7·95
60	20·2104	2·4569	0·09674	0·13497	0·23171	8·32	77·73	7·5722	7·70
90	17·5766	2·6256	0·11836	0·15069	0·26905	6·69	76·00	8·7466	7·55
120	15·8667	2·5821	0·11226	0·14439	0·25665	6·11	...	9·8838	7·70
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	4·3711	..
30	25 4688	1·8207	0·04896	0·07439	0·12335	13·98	45·00	5·9836	8·10
60	21·6069	1·9900	0·06900	0·09350	0·16250	10·85	44·61	7·0289	7·85
90	19·1226	2·0936	0·07926	0·11378	0·19304	9·15	44·16	8·0076	7·65
120	17·6400	2·1624	0·08704	0·12666	0·21370	8·14	43·40	8·6764	7·65

TABLE 21

Water hyacinth and German Basic Slag

Period in Days	Total Carbon in gms.	Total Nitrogen in gms	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	4.3711	...
30	24·2404	2·1051	0·06034	0·09436	0·15470	11·54	73·13	6·2688	7·95
60	20·1384	2·4175	0·10536	0·14127	0·24[illegible]63	8·33	74·06	7·5846	7·75
90	17·4506	2·5866	0·12467	0·15886	0·28353	6·74	72·12	8·7645	7·60
120	15·7283	2·5814	0·11986	0·15746	0·27732	6 09	...	9·8967	7·80
				DARK					
0	32·4334	1·5068	...	...	...	21·51	...	4·3711	...
30	25·4664	1·8205	0·04888	0·07445	0·12333	13·97	45·03	5·9847	8·05
60	21·6074	1·9898	0·06884	0·09346	0·16230	10·85	44·59	7·0314	7·85
90	19·1240	2·0945	0·07989	0·11327	0·19316	9·10	44·21	8·0066	7·70
120	17·6378	2·1617	0·08689	0·12688	0·21377	8·16	43·38	8·6749	7·65

TABLE 23

Water hyacinth, German Basic Slag & Tolypothrix

Period in Days	Total Carbon in gms.	Total Nitrogen in gms.	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	—	—	...	21·51	...	4·3711	...
30	24·2904	2·1258	0·05700	0·09400	0·15100	11·42	76·00	6·2700	7·90
60	20·2384	2·4460	0·09700	0·13496	0·23196	8·28	77·00	7·5866	7·70
90	17·6000	2·6344	0·12000	0·15056	0·27056	6·69	76·11	8·7586	7·60
120	15·8984	2·6000	0·11688	0·14500	0·25188	6·11	...	9·8799	7·75
				DARK					
0	32·4384	1·5066	—	—	—	21·51	...	4·3711	...
30	23·4700	1·8200	0·04888	0·07452	0·12340	13·99	45·00	5·9798	8·05
60	21·6405	1·9900	0·06900	0·09366	0·16266	10·86	44·62	7·0326	7·85
90	19·1364	2·1000	0·07954	0·11400	0·19354	9·11	44·21	8·0100	7·70
120	17·6384	2·1586	0·08724	0·12700	0·214244	8·17	13·38	8·6738	7·65

TABLE 24

Water hyacinth, German Basic Slag & Chlorella

				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	4·3711	...
30	24·3064	2·1055	0·05664	0·09333	0·14997	11·57	73·66	6·2688	7·95
30	20·2584	2·4169	0·09578	0·13366	0·21944	8·38	74·69	7·5796	7·70
90	17·6333	2·5872	0·11733	0·14774	0·26507	6·94	73·00	8·7600	7·55
120	15·9200	2·5820	0·11500	0·14480	0·25980	6·17	...	9·8879	7·65
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	4·3711	...
30	25·4658	1·8200	0·04900	0·07464	0·12364	13·97	45·00	6·0000	8·10
60	21·6000	1·9887	0·06856	0·09346	0·16202	10·88	44·56	7·0336	7·90
90	19·1286	2·0969	0·07974	0·11386	0·19360	9·15	44·19	8·0083	7·75
120	17·6400	2·1637	0·08694	0·12678	0·21372	8·12	43·41	8·6844	7·70

TABLE 25

Water hyacinth and Belgian Basic Slag

Period in Days	Total Carbon in gms.	Total Nitrogen in gms.	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	4·2055	...
30	24·2880	2·1016	0·06014	0·09394	0·15408	11·56	73·00	6·0946	7·90
60	20·2069	2·4035	0·10336	0·14000	0·24336	8·40	78·34	7·2468	7·70
90	17·5190	2·5853	0·12195	0·15736	0·27931	6·80	72·40	8·3746	7·65
120	15·8085	2·5796	0·11999	0·15702	0·27701	6·13	...	9·2947	7·75
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	4·2055	...
30	25·5000	1·8189	0·04874	0·07438	0·12312	14·01	45·00	5·7684	8·00
60	21·6644	1·9806	0·06804	0·09266	0·16090	10·97	44·41	6·7886	7·85
90	19·1684	2·0910	0·07906	0·11294	0·19200	9·21	44·04	7·6689	7·70
120	17·7006	2·1400	0·08549	0·12578	0·21127	8·27	43·00	8·3106	7·60

TABLE 26

Water hyacinth, Belgian Basic Slag & Anabeana

				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	4·2055	...
30	24·3300	2·1066	0·05684	0·09314	0·14998	11·53	74·07	5·0886	7·85
60	20·2774	2·4125	0·09644	0·13384	0·23028	8·40	74·51	7·2300	7·65
90	17·6384	2·5983	0·11833	0·15000	0·25833	6·81	73·78	8·3388	7·60
120	15·9700	2·5816	0·11300	0·14400	0·25700	6·19	...	9·2800	7·70
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	4·2055	...
30	25·5000	1·8192	0·04868	0·07440	0·12308	14·00	45·01	5·7700	8·00
60	21·6700	1·9800	0·06794	0·09266	0·16060	10·98	44·40	6·7900	7·85
90	19·1700	2·0933	0·07924	0·11283	0·19207	9·23	44·06	7·6700	7·70
120	17·7006	2·1398	0·08496	0·125690	0·21065	8·27	43·00	8·3088	7·65

TABLE 27

Water hyacinth, Belgian Slag & Tolypothrix.

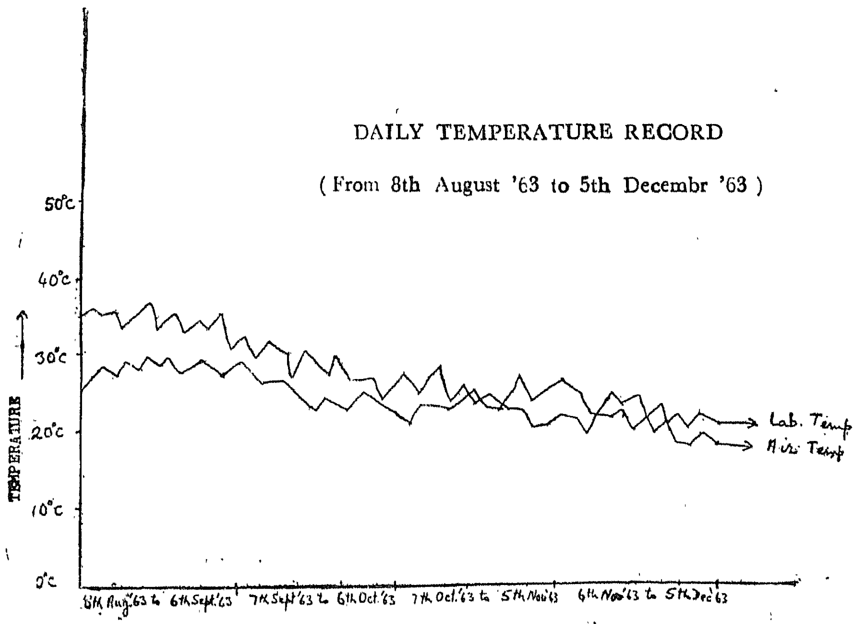
Period in Days	Total Carbon in gms.	Total Nitrogen in gms.	NH_3—N in gms.	NO_3—N in gms.	Available Nitrogen in gms.	C/N Ratio	Efficiency	Available P_2O_5	pH of the Mix.
				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	4·2055	...
30	24·3384	2·1078	0·05596	0·09288	0·14884	11·51	74·23	5·0866	7·85
60	20·2994	2·4148	0·09538	0·13244	0·22782	8·38	74·84	7·2286	7·60
90	17·6600	2·6006	0·11694	0·14888	0·26582	6·79	74·05	8·3400	7·55
120	15·9984	2·5834	0·11288	0·14333	0·25621	6·20	...	9·2788	7·70
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	4·2055	...
30	25·5123	1·8200	0·04874	0·07440	0·12314	14·00	45·11	5·7693	8·00
60	21·6666	1·9844	0·06813	0·09300	0·16113	10·95	44·43	6·7800	7·80
90	19·2000	2·0896	0·07906	0·11266	0·19172	9·22	44·04	7·6668	7·65
120	17·6974	2·1400	0·08556	0·12584	0·21140	8·26	43·00	8·3000	7·60

TABLE 28

Water hyacinth, Belgian Basic Slag & Chlorella

				LIGHT					
0	32·4384	1·5066	...	...	...	21·51	...	4·2055	...
30	24·3400	2·1016	0·05548	0·09300	0·14848	11·59	73·50	5·0844	7·85
60	20·3104	2·4046	0·09388	0·13168	0·22556	8·45	74·22	7·2238	7·65
90	17·6884	2·5862	0·11564	0·14688	0·26252	6·85	73·24	8·3246	7·50
120	16·0084	2·5800	0·11300	0·14000	0·25300	6·20	...	9·2655	7·70
				DARK					
0	32·4384	1·5066	...	...	...	21·51	...	4·2055	...
30	25·5000	1·8169	0·04868	0·07396	0·12264	14·12	44·97	5·7700	8·00
60	21·6723	1·9800	0·06788	0·09278	0·16066	10·98	44·41	6·7786	7·80
90	19·2038	2·1000	0·07886	0·11304	0·19190	9·14	44·08	7·6659	7·65
120	17·6884	2·1444	0·08574	0·12569	0·21143	8·25	43·11	8·2968	7·55

From the foregoing results we come to the conclusion that the oxidation of carbon in Water-hyacinth (Eichornia crassipes) with or without phosphate used in the form of different basic slags, is more pronounced in the exposed condition than the covered state, but this carbon oxidation of the exposed sets is checked a



little by the algae. The efficiency of nitrogen fixation is greater in the exposed sets than in the covered ones and there is a further increase in the afficiency in presence of alage. The rate of increase of nitrogen in the system is fast in the first thirty to sixty days but slowly falls off as the time of exposure increases. This further supported by Dhar according to him that the carbon of organic matter is finally oxidised in the system to carbon di oxide and a considerable amount of energy is liberated according to the following equation:—

$$C_6H_{12}O_6+6O_2\rightarrow6CO_2+6H_2O+676 \text{ K Cals.}$$

This energy thus evolved is utilized in the endothermic reaction by which the atmospheric nitrogen is fixed. The mechanism of the nitrogen fixation process seems to be through the decomposition of water molecule into atomic hydrogen and hydroxyl radical by absorption of energy obtained from the oxidation of carbonaceous matter. Light plays an important role in this process of nitrogen fixation as it is absorbed in the system and thus supplies additional energy for these reactions to go on.

But this process of nitrogen fixation is always counteracted by the nitrogen loss taking place simultaneously, because as soon as some nitrogen is fixed in the system the process of nitrification starts. During the nitrification as unstable substance, ammonium nitrite, which readily decomposes into free nitrogen gas and water, is formed. This is responsible for the observed fact that the efficiency of nitrogen fixation declines with lapse of time.

Our foregoing results further show that the anabeana or Tolypothrix containg sets have more nitrogen than the without algae or with the chlorella. This is supported the view of Watanabe, Allen, Tamiya and others. According to them that anabeana or tolypothrix fixes the atmospheric nitrogen in the system and on the other hand the chlorella does not fix any nitrogen from air. The effect of the algae and the different basic slags on the nitrogen fixation is in the followings orders:—

German Basic Slag+Tolypothrix or Anabeana > Germana Basic Slag+Chlorella > Belgien Basic Slag+Tolypothrix or Anabeana > German Basic Slag > Belgien Basic Slag+Chlorella > Belgien Basic Slag > Tata Basic Slag+Tolypothrix or Anabeana > Tata Basic Slag+Chlorella > Tata Basic Slag > Kulti Basic Slag+Tolypothrix or Anabeana > Kulti Basic Slag+Chlorella > Kulti Basic Slag > Durgapur Basic Slag +Tolypothrix or Anabeana > Durgapur Basic Slag+Chlorella > Durgapur Basic Slag > Rourkela Basic Slag+Tolypothrix or Anabeana > Rourkela Basic Slag+Chlorella > Rourkela Basic Slag > Tolypothrix > Anadeana > Chlorella > Organic Matter alone.

On the other hand the results further emphasized the sensitivity of the three algae in the following order:—

Chlorella > Tolypothrix > Anabeana

The data further concluded that the Tolypothrix is better out of all the three algae from the nitrogen fixation point of view, Anabeana is the second and Chlorella doesnot fix any nitrogen in the system. These algae do not show any growth in any set of dark.

The temperature of the place, where the experiment was conducted, did not alter much and always below 30° C, on the other hand the atmospheric temperature shows greater fluctuations and sometimes it rishes above 38° C and sometime fall down below 16° C. Both the temperature shown by the graph on the previous page.

It is evident from our results that on the addition of basic slags to the waterhyacinth, rapid growth of algae takes place. This is due to the fact that on adding basic slag, the solution of the system becomes rich in calcium, iron, phosphates and also the trace elements which helps in the growth of algae. Moreover basic slag may act as buffers in decreasing the acidity of medium and help carbohydrate metalbolism.

It has been observed in all these experiments of algae that available nitrogen always less than those sets which contains no algae. The order is in the following way:—

Tolypothrix > Anabean > Chlorella

But the total nitrogen was same or higher in compersion the sets which contains no algae. This is due to the fact that algae eat the availables nitrogn and formed proteins by the helps of CO_2 and nitrogen from the air. It has been found that the carbon nitrogen ratio of the phosphated and light exposed composts is always less than the unphosphated and the covered composts. This simply explains that the production of nitrogenous-compounds by the fixation of atmospheric nitrogen per unit weight of carbon oxidised is higher in the phosphated and light exposed sets.

Upto a certain period, the pH of phosphated and unphosphated sets, is much smaller in light receiving sets than covered ones. The fall in pH may be accouned for the liberation of more acids in light than in covered sets as a result of

enhanced decomposition of organic matter and the oxidation of nitrogeneous compounds. The pH of algae sets is slightly smaller than the sets containing no algae. This may be due to the excess of CO_2 absorved by the system from air. But after some interval of time the pH of the phosphated and unphosphated exposed composts begins to rise. This rise in pH is more in light than in dark. This is likely due to the break down of acids and light appears to enhance this process causing more increase in exposed sets than in covered.

The results further emphasized that anabeana, chlorella or tolypothrix does not show any remarkable effect on the availability of the phosphate in the system. The available P_2O_5 is always equal or slightly less from those sets which contain no algae.

Hence we have reached the conclusion that water hyacinth compost which is rich in nitrogen & potash, not only supplies the major plant nutrients but also supplies the trace elements which are present in basic slag poorer or richer in phosphate content. This will be an excellent balanced compost in tropical soils. In making the compost, algae does not play an important part in fixing up the atmospheric nitrogen.

Similar composting experiments with the same organic matter performed in large and small wodden boxes, show that even after 250 days exposure to sunlight, these system containing algae have very slight effect on nitrogen fixation likely to be due to increase in the temperature or shortage of excess water. The experiment show that in wheat growing, these algae or compost of algae did not show any effect in the quality or quantity of wheat grains or wheat straw. Hence we have concluded that these algae or compost of algae do not fruitful for wheat growing.

REFERENCES

Frank, B., *Ber. deut. botan. Ges.* 7, 34 (1889).

Drewes, K., *Zentr. Bakteriol Parasitank* 11, 76, 83 (1928).

Brotels, H., *Arch. Mikrobiol*, 11, 155 (1940).

Fogg, G. E., *New Phytol.* 43, 164 (1944).

Fogg, G. E, *Proc. roy. Soc. B.* 139, 372 (1952).

Fygg, G. E., *New Biology.*, 18, 52 (1955).

Fogg, G. E., *Ann. Rev. Plant. Physiol.*, 7, 51 (1956).

Fogg, G. E., *Biochin et Biophys. Acta.*, 30, 209 (1958).

Watanabe, A., *Arch. Biochem. and Biophys.* 34, 50 (1951).

Watanabe, A., *Bot. Mag. Tokyo*, 69, 530 (1956).

Watanabe, A., *J. gen appl. Microbiol, Tokyo*, 5, 21, & 85 (1959).

Herisset, A., *Bull. Soc. Chim. biol.* 34, 532 (1952).

Tamiya, H., *Ann. Rev. Plant. Physiol*, 8 309 (1957).

Allen, M. B., *Scientific Monthly*, 83, 100 (1956).

Allen, M. B., & Aron, D. L., *Plant Physol.*, 30, 366 (1955).

Singh, R.N., *Verh. int. Ver. Limnol*, 12, 831 (1955).

Singh, N. R., Blue Green Algae in Nitrogen Economy of Indian Agriculture, I. C. A. R. Publication (1961).

Dhar, N. R., Presidental Address, Nat. Acad. Sci., India (1953).

Dhar, N. R., *Kungi, Lant. Annalar.* 21 (1954).

Dhar, N. R., *Indian Agriculturist Vol.* 3 (1959).

Dhar, N. R., *Nat. Acad. Sci., India, Vol.* 33, p. 16-24 (1963).

Russel, E. J., Soil Conditions & Plant Growth, Lond (1953).

Piper. C. S., Soil and Plant Analysis (1950).

Vogel, A. I., Quantitative Inorganic Analysis (1959).

STUDIES ON THE BLUE-GREEN ALGAE AS GREEN MANURE IN JAPAN

By

ATSUSHI WATANABE

Biologieal Laboratory, Seijo University, Seijo-cho, Setagaya-ku, Tokyo, Japan

ABSTRACT

In this paper the distribution and physiological behaviour of the nitrogen fixing blue green algae in Japan and in the South & East Asia has been recorded.

The yield of paddy after inoculation with Tolypothrix is greater by 15% in well-drained paddy fields and by 25% in badly-drained paddy fields.

It has been claimed that nitrogen fertility of the soils and yield of paddy in Japan can be greatly improved by the application of algal inoculation.

The ability of some blue-green algae to fix atmospheric nitrogen and its possible agricultural implications have been well substantiated and documented. The present review outlines some of the fundamental and applied aspects of the investigations carried out in Japan with special reference to the application of the blue-green algae as green manure to the paddy fields.

Nitrogen fixation in flooded paddy soils by blue-green algae ;

An abundant occurrence of blue-green algae is a common phenomenon in flooded paddy fields and the possible role of these alage in the nitrogen recuperasion of the soils was studied by Shioiri et al (1944). Under laboratory conditions when the soils were kept waterlogged, blue-green algae like *Nostoc and Oscillatoria* grew abundantly within few days resulting in a significant increase in the nitrogen content of the soils. The application of lime and phosphate was found to accelerate the growth of these blue-green algae, as was reported earlier by De in India. Similar observations were made by Okuda and Yamaguchi (1952). However these workers could observe the effect of inoculation only in four out of twelve samples On the basis of their observations Okuda and Yamaguchi (1952) classified the soils into four groups, viz. (a) soils in which nitrogen fixing algae grew naturally with considerable fixation of nitrogen without any treatment; (b) soils in which algae grew and fixation increased only when they were inoculated with these organisms; (c) soils in which the inoculation of algae was effective only when supplied with lime and phosphate and (d) soils in which nitrogen fixation was poor in spite of all these treatments. While the inoculation of blue-green algae will not have any additional effect in the first type of soils, the inoculation will be effective in the second and third types of soils. The fourth type of soil needs further investigations.

The practice of successive lime application is said to impoverish the soils. However, the lime application was found to accelerate the growth of blue-green algae which in turn fixed sufficient nitrogen thereby compensating the loss induced by lime application (Mitsui, 1960).

Distribution of nitrogen fixing blue-green algae in Japan and in South and East Asia :

With a view to evaluate the practical significance of the nitrogen fixing blue-green algae, the distribution pattern of these algae in the rice fields of Japan was studied by Okuda and Yamaguchi (1956a, b). From an analysis of 117 soil samples from various parts of Japan, they concluded that the blue-green algae had a wide distribution and particularly the nitrogen fixing forms were abundant. Although the soil texture or its organic matter content had no bearing on the growth of these algae, there was a correlation between the pH and the phosphorous content, of the soil and the occurrence of blue-green algae. Wadanabe (1959) extended his studies to the soils from various parts of South and East Asia. Out of 851 soil samples critically examined by him, 16 species were found to be capable of fixing atmospheric nitrogen. His investigations showed that while the blue-green algae were abundant in tropical and semitropical regions like Java, Sumatra, Borneo, the Phillipines, Malayasia, Indo-China, Siam, Burma, Hainam, Formosa and Southern China, their occurrence in Japan, Northern China, Manchuria, Korea and Sakhal in was comparatively less abundant. Most of the blue-green algae encountered in these investigations belonged to the genera *Tolypothrix, Nostoc, Cylindrospermum, Calothrix, Anabaena, Plectonema, Anabaenopsis* and *Schizothris. Tolypothrix* and *Nostoc* showed a wide range of distribution. The nitrogen fixing capacity of *Tolypothrix tenuis* from Borneo soils, *Calothrix brevissima* from Palau islands, *Anabaenopsis circularis* from Sumatra and *Nostoc* sp. from Java was examined under bacteria-free conditions and it was found that the amount of total nitrogen fixed by these forms per 100 ml of the medium in 20 days was 9.6 mg, 5.2 mg, 3.4 mg and 3.1 mg respectively (Watanabe, 1959).

Watanabe (1963) also investigated the nitrogen fixing capacity of symbiotic blue-green algae, using N^{15} and found that *Nostoc punctiforme* from lichens, *N. sphericum* from liverworts and *N. cycadeae* from cycads were able to fix nitrogen both independently as well as in symbiotic association with their hosts.

Physiological and Biochemical researches on nitrogen fixing blue-green algae :

The growth of the Borneo strain of *Tolypothrix tenuis* as influenced by light intensity, temperature and the composition of cultural medium was studied by Ukai et al (1958). On the basis of these investigations an improved medium for growth experiments and one for economical culture of the alga were suggested, In the latter medium, Cubic Nitre and calcium superphosphate were used as the nitrogen and phosphate source, while 'brine' from the salt industry was used as the source of magnesium. Okuda and Yamaguchi (1962) suspecting the deficiency of some minor element as the possible cause for the failure of the growth of autochthonous algae, examined the molybdenum requirements of a Japanese strain of *Tolypothrix tenuis* and found an optimum concentration of 10^{-2} ppm Mo for the efficient growth of the alga, irrespective of the nitrogen source whether in the molecular form or in a nitrate form. However, in the presence of ammonium sulfate, urea and mono sodium glutamate, no growth responses for molybdenum were observed.

The heterotrophic nutrition of *Tolypothrix tenuis* (from Borneo soils) was critically examined by Kiyohara et al (1960), who found that a simultaneous supply of casamino acids and glucose as the nitrogen and C-and/energy source was required

for the maximum growth under these conditions. Though the alga could assimilate exogenous glucose as such in light converting it into cellular carbohydrates, in dark it could use glucose only as a fuel substance and not as a building material for cellular substances (Kiyohara et al 1962).

Fujiwara and Okutsu (1959a) working with *Nostoc spongiaeforme* suggested an improved culture medium for the rapid and abundant growth of this alga. They also contributed to the understanding of the influences of pH, illumination and aeration on the growth of this alga (Fujiwara and Okutsu, 1959b).

Microorganisms are known to excrete soluble organic substances during their growth phase, some of which are biologically important, *Tolypothrix tenuis, Calothrix brevissima, Anabaenopsis circularis* and a species of *Nostoc* were examined from this point of view by Watanabe (1951) and it was found that although many amino acids were detected in the algal body, the external medium was poor in amino acids or lacked them. However, *Calothrix brevissima* alone was found to excrete aspartic acid, glutamic acid and alanine (Watanabe, 1951).

Interesting as it is, Okuda and Yamaguchi (1960) reported the synthesis of vitamin B_{12}-active substance by *Tolypothrix tenuis,* the yield of which came upto about 700*r*%. The growth of the alga and the production of vitamin B_{12}-active compounds were found to be promoted by the addition of cobalt salt, but not by nitrogen or carbon sources.

Mass culture of Blue-Green Algae as 'seeding' material and methods of preservation over a long period :

For the wider application of the nitrogen fixing blue-green algae in the agneral agricultural practices as green manure, it is mandatory to grow these legae in large quantities. The methods followed by Watanabe (1959b) to obtain in a large quantity of Tolypothrix tenuis involved (a) a preliminary shaking culture using ordinary culture flask, (b) a stirring culture in a tank and (c) an outdoor culture of 'closed circulation system' using a large flat bag made of sheeting of polyvinyl chloride. The maximum growth rate in the tank culture was 0.2 g/l/day and 7.9 g/m²/day in the outdoor culture. To economize the production cost Watanabe et al (1959b) used hot spring water and natural gas (methene) as the heat and carbon dioxide sources. The yield of alga under this device amounted to 6.4g (dry weight) /m²/day which in terms of tons worked out to 7 tons of alga per year.

Equally important is the preservation of these algae as 'seeding' materials, since these organism putrefy readily in a wet state. Watanabe (1959c) suggested the lyophilization techinique and also devised a method to culture them on moist porous volcanic gravels, commonly known in Japan as *Kanuma-tsuchi.* In both methods the alga retained its capacity for revival for a period of 2 years, although in the former method the percentage of viability was reduced by about fifty percent.

Effect of Nitrogen Fixing Blue-Green Algae on the Growth and Crop Yield of Rice :

The effect of Tolypothrix tenuis, Calothrix brevissima, and Anabaenopasis circularis on rice plants was studied by Watanabe et al (1951) who reported a positive effect of Tolypothrix tenuis on the growth of rice plants. This beneficial effect was discernible both in well-drained and badly-drained paddy fields (Watanabe et al 1951) (Table 1).

TABLE 1

Effect of Tolypothrix tenuis on the yield of rice (Watanabe et al 1951).

	Yield (bushels/acre)		Percentage increase
Well-drained paddy field	Uninoculated	44·9	
	Inoculated with Tolypothrix	51·8	15
Badly-drained paddy field	Uninoculated	30·3	
	Inoculated with Tolypothrix	37·8	25

The effect of Tolypothrix tenuis (from Borneo soils' on the growth and yield and rice crop was examined in about forty field experiments conducted at various parts of Japan from 1951 to 1956. Konishi and Seino (1961) conducted inoculation experiments at the Hokuriku Agricultural Experimental Station, for a period of six years (1949-54) followed by two years experiment to study the residual effect of the inoculation. The inoculation was found to be significantly effective when supplemented with calcium carbonate. (Table 2)

TABLE 2

Effect of inoculation of Tolypothrix tenuis on the yield of rice.

	Treatment	Yield of brown rice/annum (Kg/10a)	
		1949-54	1955-56 (Residual effect)
Without $CaCO_3$	Uninoculated	337·2	338·2
	Inoculated with alga	342·1	329·1
With $CaCO_3$	Uninoculated	371·5	382·9
	Inoculated with alga	388 5	384·5

Hosoda and Takata (1955) from their field experiments conducted at Tottori University concluded that the effect of the inoculation with Tolypothrix tenuis was almost similar to the effect of fertilizing with 71.8 Kg/hectare ammonium sulfate as the additional fertilizer. The nitrogen content of both straw and rice increased by 10.9% and 3.2% respectively. The investigations carried out at the Shikoku Agricultural Experiment Station showed that the yield of rice in the field inoculated with Tolypothrix tenuis registered a progressive increase over the uninoculated control plots by 5, 10, 15, 20, 23% respectively over a period of five consecutive years. Ogiwara (1952-6), Nishigaki et al (1951; 1952-56), Shiroshita and Takahashi (1952-5), Todo (1952-6)), Saito (1952-6) and Tanaka (1952-6) carried out series of field experiments in various Agricultural Experimental Stations at Fukuoka, Saitama, Aichi, Chiba and Wakayama Prefectures from 1952 to 1956. Table 3 summarizes the

TABLE 3

Experimental farm	Tester	No. of replication	Area of one lot (acre)	Percentage increase in crop yield				
				1st year	2nd year	3rd year	4th year	5th year
Fukuoka	T. Ogiwara	6	1/12000 (Earthen pipe)	—4	17	56	44	11
Saitama	S. Nishigaki M. Shibuya S. Sakai and C. Okada	6	1/8000 (Earthen pipe)	2	15	30		
Tottori	K. Hosoda and H. Takata	4	1/240 (Paddy field)	8	10	18		
Shikokn	T. Hirano	3	1/600 (Paddy field)	5	11	15	20	23
Kanto	T. Shiroshita and K. Takahashi	3	1/400 (Paddy field)	0	3	11		
Kokuriku	C. Konishi and K. Seino	4	1/200 (Paddy field)	5	2	8		
Aichi	M. Todo	3	1/240 (Paddy field)	4	6	3		8
Chiba	K. Saito	4	1/240 (Paddy field)	—1	10	2	10	8
Wakayama	H. Tanaka	4	1/240 (Paddy field)	—1	—2	—7	4	3
Average				2·0	8·0	15·1	19·5	0·6

results, obtained by these workers. Despite some erratic trends seen in these results, the overall picture shows on an average an increase in the yield of rice by 2% in the first year, 8% in the second year, 15.1% in the third year, 19.5% in the fourth year and 10.6% in the fifth year. In the experiments conducted at Wakayama and Aichi Prefectures, the inoculation of the alga failed to establish itself, the causes of which are still unknown. However, the inoculation with a Japanese strain of Tolypothrix tenuis and Nostoc spongiaeforme had no significant effect on the rice crop although the nitrogen absorption by the crop was slighty enhanced (Yamaguchi, 1961).

Contribution of the algal inoculation to the potential nitrogen fertility of the soils :

Using the methods of Shioiri and Aomine (1940) Ogiwara (1952-6) observed that the nitrogen fertility of the soil inoculated with the algae was always higher than the uninoculated soils. Table 4 summarizes the results of these observations (Watanabe, 1962).

TABLE 4

Change in the potential fertility of soil nitrogen due to inoculation with Tolypothrix tenuis (Watanabe, 1962).

Treatment	Amounts of ammonification mg/100 g air dried soil					
	1st year		3rd year		4th year	5th year
Death in cm	0-2	2-10	0-2	2-10	0-2	0-2
Control	13·7	5·4	15·4	5·3	12·6	12·0
Inoculated	14·3	7·1	20·0	6·1	13·1	12·6

Confirmation of the nitrogen fixation by Tolypothrix tenuis under field conditions came from the N^{15} experiments of Nishigaki et al (1951; 1953) which showed a fixation of about 22.5 Kg of nitrogen per hectare by the alga and an absorption of about 4.8Kg of nitrogen per hectare by the crop.

Since the final utilization of the algal nitrogen by the crop depends on its conversion by the bacterial activity into assimilable from, Watanabe, and Kiyohara (1960) investigated this aspect, and showed that a certain strain of Bacillus subtil is converted about 40% of the algal nitrogen into ammonia within 10 days of incubation.

Methods to implement soil inoculation and to ensure the establishment of the inoculum in the paddy fields :

The problems concerning the success of algal inoculation and the attempts to reori-ntate the local soil microflora in favour of the beneficial organisms will naturally vary from region to region. The investigations aimed in this direction in Japan suggest that the following considerations have proved efficaceous.

(1) Sprinkling of lime powder on the paddy water prior to seeding helps in lowering the acidity of the water and favours the growth of the inoculum.

(2) Regulation of the inflow of cold irrigation water facilitates in maintaining the paddy field water warm.

(3) Application of phosphatic fertilizers and suitable amounts of molybdenum favour the growth of the inoculum and its fixation.

(4) The contaminating organisms like daphnids can be prevented by the suitable application of pesticides like folidol or Parathion (Watanabe et al 1955a, 1956b). Similar application of Parathion eliminates small animals like Bulimus, Branchinella, Leptestheria and others (Hirano et al 1955). Ishizawa (1964) found that 100 ppm of pentachlorophenol (PCP) suppressed the green algae without any deleterious effect on the blue-greens.

Prospects of utilizing the blue-green algae as green manure :

Though the beneficial effects of algal inoculation to increase the crop yield and soil fertility have been demonstrated amply, an intensive and concentrated approach on the following lines in many other countries will remove many of the obstacles encountered at present and help to harness this natusal resource more efficiently in our general agricultural practices.

(*a*) An extensive search is needed to select and isolate blue-green algal strains having more powerful ability to fix atmospheric nitrogen than the known strains available at present.

(*b*) Strain which could propogate easily and speedily under field conditions should receive more attention, though their nitrogen fixing capacity is slightly lower.

(*c*) Since the natural soil microflora are composed of many types of organisms attempts should be made to evaluate the combined contribution of these organisms together with the blue-green algae. Similarly information about the combined effects of many types of nitrogen fixing blue-green algae is needed.

(*d*) Economic methods of culturing these algae on a mass scale and the methods to preserve them in an active state over a long period for distribution are to be worked out in various regions. The porous phospphate fertilizer, a kind of artificial phosphate fertilizer, as the base for the 'seeding' material is useful, since the phosphatic base can also be used by the crop.

(*e*) Investigations on the heterotrophically culturing these algae should be intensified.

It is needless to stress the great potentialities of these blue-green algae serving as green manure in the Indian paddy fields. The prevalent high atmospheric temperature, the general alkalinity of the field water and low nitrogenous manuring in India offer ideal opportunities to exploit thege algae to the best advantage.

REFERENCES

Fujiwara, A., and Okutsu, M. (1959a) : Tohoka *J. Agri. Res. Japan*, 10;97.

Fujiwara, A., and Okutsu, M. (1959b) : *Ibid.* 10:321.

Hirano, T., Shiraishi, K., and Nakano, K. (1955): *Bull. Shikoku Agr. Expt. Stn.*, 2:121 (in Japanese with english summary).

Hosoda, K., and Takata, H. (1955) : *Trans. Tottori Soc. Agr. Sci.*, 10:1 (in Japanese with english summary).

Ishizawa, S. (1964) : *J. Sci. Soil. & Manure*, No. 10 (an extra report) (in Japanese).

Kiyohara, T., Fujita, Y., Hattori, A., and Watanabe, A. (1960) : *J. Gen. Appl. Mierobiol.*, 6 : 176.

Kiyohara, T., Fujita, Y., Hattori, A., and Watanabe, A. (1962) : *Ibid.* : 8 : 165.

Konishi, C., and Seino, K. (1961) : *Bull. Hokuriku Agr. Exp. Stn.*, 2:41 (in Japanese with english summary.

Mitsui, S., (1960) Inorganic Nutrition Fertilization and Soil Amelioration for Lowland Rice. Pub. Yokendo Ltd., Japan.

Nishigaki, S., Shibuya, M, and Kodaira, K. (1951) : *J. Sci, Soil & Manure*, 22:69 & 70 (in Japanese).

Nishigaki, S., and Shibuya, M. (1953) : *Ibid.* 23 : 150 (in Japanese).

Nishigaki S., and Shibuya, M. (1954-55) : *A. Rep. Dept. Chem. Nat. Inst. Agr. Sci.*, Japan (in Japanese).

Nishigaki S., Shibuya, M. Sakai, S, and Okada, C. (1952-56) : Res. Data on Blue-green Algae. Saitama Agr. Exp. Stn. (in Japanese).

Ogiwara, T. (1952-56) : Res. Data on Blue-green Algae. Fukuoka Agr. Exp. Stn. (in Japanese).

Okuda, A., and Yamaguchi, M. (1952a) : *Mem. Res. Inst. Food. Sci. Kyoto Univ.*, 2 : 1.

Okuda, A., and Yamaguchi, M. (1952b) : *Ibid.* 4 : 1.

Okuda, A., and Yamaguchi, M. (1955) ; *Soil & Plant Food*, 1 : 102.

Okuda, A., and Yamaguchi, M. (1956a) : *Ibid.* 2:4..

Okuda, A., and Yamaguchi, M. (1956b) : VI *Cong. Int. Sci. Sol* (Paris), p. 521

Okuda, A., and Yamaguchi, M. (1960) ; *Soil & Plant Food*, 6 : 76.

Okuda, A., and Yamaguchi, M. and Nioh, I. (1962) *Soil Sci. & Plant Nutrition*, 8 : 35.

Saito, K., (1952-56) : Res. Data on Blue-green Algae. Chiba Agr. Exp. Stn. (in Japanese).

Shiroshita, T., and Takahashi, H. (1952-55) : Res Data on Blue-green Algae. Kanto Higashiyama Agr Fxp. Stn. (in Japanese).

Shioiri, M., and Aomine, S. (1940) : Bull. Imp. Agr. Exp. Stn. Japan (an extra report) (in Japanese).

Shioiri, M., Hatsumi, T, and Nishigaki, S. (1943) : *J. Sci. Sail & Manure*, 17:288.

Shioiri, M., Hatsumi, T., and Nishigaki, (1944) : *Ibid.* 18:59.

Tanaka, H. (1952-56) : Res. Data on Blue-green Algae. Wakayama Agr. Expt. Stn. (in Japanese).

Todo, M. (1952-56) : *Idem.* Aichi Agr. Exp. Stn. (in Japanese).

Ukai, Y., Fujita, Y., Morimura, Y., and Watanade, A. (1958) : *J. Gen. Appl. Microbiol.*, 4:163.

Watanabe, A. (1950) : Misc. Rep. Res. Inst. Natl. Resources, Tokyo. 17-18:61 (in Japanese).

Watanabe, A. (1951a) : *Arch. Biochem. Biophys.*, 34:50.

Watanabe, A, (1956) : *Bot. Mag. Tokyo*, 69: 530 (in Japanese with english summary).

Watanabe, A, (1959) : *J. Gen. Appl. Microbiol.*, 5:21.

Watanabe, A. (1959b) : *Ibid.* 5:85.

Watanabe, A. (1959c) : *Ibid.* 5:153.

Watanabe, A. (1962) : *Ibid.* 8 : 85.

Watanabe, A., Nishigaki, S., and Konishi, C. (1951) : *Nature*, 168:748.

Watanabe, A., Ito, R and Sasa, T. (1955a) : *J. Gen. Appl. Microbiol.*, 1:137.

Watanabe, A., Ito, R., and Sasa, T. (1955b) : *Ibid.* 1:190.

Watanabe, A., Hattori, A., Fujita, Y., and Kiyohara, T. (1959) : *Ibid.* 5:51.

Watanabe, A. and Kiyohara, T. (1960) : *Ibid.* 5:175.

Watanabe, A. and Kiyohara, T. (1963) : In Studies on Microalgae and Photosynthetic Bacteria. Plant & Cell Physiol., (a special issue), p. 189.

Yamaguchi, M. (1961) : Studies on the Atmospheric Nitrogen Fixation by Blue-green Algae in Paddy soils. Ph. D. Thesis, Kyoto University.

ALGAL CULTURE IN ORGANIC WASTES

By

A. C. GAUR

*Technological Institute, Northwestern University, Evanston, Illinois**

ABSTRACT

An account of the work done on the treatment of organic wastes in stabilisation ponds by growing algae has been discussed. Experimental results on the possibility of growing filamentous algae with bacteria in pond water containing organic wastes show that *Oscillatoria*, which is a filamentous blue green alga, can grow well in sewage.

The natural process of stabilising organic wastes by bacterial oxidation and of producing oxygen by algae through photosynthesis are fundamental in the treatment of organic wastes in stabilization ponds. Field observations and research studies have shown that under proper conditions several species of algae may grow vigorously in waste waters containing moderate concentrations of organic materials.

It has been suggested by Caldwell (1) and Myers (2) that in sewage lagoons or oxidation ponds the stabilisation of organic matter effected by aerobic bacteria is greatly aided by the simultaneous proliferation of algae. It is well known that the principal product of aerobic bacterial oxidation of organic matter are CO_2, NH_3, and water which, except for the additional requirement of light energy, are identical to the principal requirements for algal photosynthesis. Thus, in theory, the decomposition of organic matter by bacteria, at the same time that new organic matter is synthesized by algae, provided the light is available as the energy source. Under such circumstances the efficiency of oxygen utilization is greatest because oxygen is used as soon it is formed. The cycle of photosynthetic oxygenation is shown in the following figure (1). Organic matter entering the system as sewage or other kind of organic waste is oxidised by sewage bacteria utilising oxygem released by algae. The algae, utilising solar energy are simultaneously synthesizing organic matter from hydrogen liberated in their chlorophyll pigment and from CO_2 and NH_3 produced by bacteria. Although this entire reaction may occur in a closed system, some CO_2 is normally drawn into the cycle from the outside atmosphere and excess oxygen may be lost.

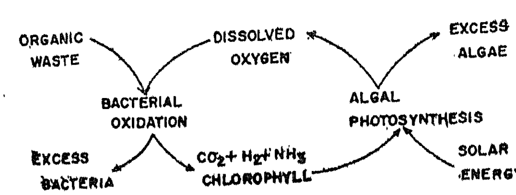


FIG. 1.

The feasibility of such a symbiotic relationship has been extensively studied by Gotaas, Oswald and coworkers (3 to 11) using oxygen ponds specially

* *Present address.*—Soil Microbiologist, Division of Microbiology, I.A.R.I., New Delhi-12.

designed for the purpose. The ponds are equiped with automatic devices to control the rate of sewage inflow, liquid depth, and the rate of recirculation of effluent to seed the incoming sewage. The species of algae growing in the ponds are *Chlorella, Scendesmus, Chlamydomonas*, and *Euglena*, among which *Chlorella* is dominant in most cases (3). The yield of algae as well as the degree of purification (or lowering of BOD value) of sewage water brought about by Algal-bacterial simultaneous growth depends on various such factors such as (1) *Detention period of sewage in the pond*. The detention period or time the liquid is held in continous flow growth tanks, may be defined as $D = \frac{V}{v}$ where D is detention period expressed in days, V is the volume of the culture, and v is the daily feed or withdrawal volume (2) *Depth of the liquid* (3) *Original* BOD *value* of sewage in pond (sewage strength) - Biochemical oxygen demand is defined as the amount of oxygen used by microorganisms in stabilising organic matter under aerobic conditions usually reported in milligrams per litre, consumed during an initial five day period at some constant temperature. BOD is used as an index of the organic content of the sewage as it is widely used mass bioassy method for determining the strength of the sewage or its organic nutritional character for bacterial growth. Values are reported for 5 day incubation at 25°C. (4) *Bacterial and algal species*. (5) *Amount of solar energy received* and (6) *Temperature*. A rather simplified formulation relating to these diverse factors have been proposed by Gotaas et al. (10, 11). According to these calculation, a suitable detention period is from one and one-half to four days depending on the season and local climatological conditions and suitable pond depth is from six to twenty centimeters depending mainly on the sewage strength (3).

From their experimental results, Gotaas et al. inferred that a photosynthetic oxygenation pond when properly designed and operated will bring about a BOD removal of as much as 70 to 80%, a light energy utilisation of as much as 5 to 8 % (9) and a production of algae in the amount of 30 to 35 tons per acre per year at Richmond, California or 42 tons per acre at Phoenix, Arizona (10, 11).

One technical problem which seems important in determining the economic feasibility of the project is the method of harvesting algal cells from the sewage. Whereas the mass culture of green algae discussed before the density of algae may be raised to a level as high as several grams per litre. There is rather sharp upper limit of algal density attainable in the culture in the sewage. The limit is only about 300 mg. per litre in moderately strong domestic sewage (10), which is principally determined by the carbon and nitrogen content of the sewage. Present day centrifuge equipment does not appear to be satisfactory from an economic point of view for separating the algae from a liquid of such low density. To overcome the difficulty Gotaas et al. (2) proposed a method of precipitating algal cells with alum as a coagulant. The possibility of separating and recovering aluminum floc on a large scale is problem which must await further experimentation.

Gaur, Pipes and Gotaas (12) carried on experiments on possibility of growing satisfactorily filamentous algae with bacteria in water containing organic wastes, which might be harvested more economically than unicellular algae.

The preliminary experiments with filamentous algae like *Ulothrix*, and *Hormidium* were disappointing, although some growth of *Hormidium* was obtained. However, a strain of *Oscillatoria* a filamentous blue-green alga which grows quite well in sewage was obtained and the following conclusions were drawn:—

This strain of *Oscillatoria* grows well under laboratory conditions in sewage and other organic wastes producing yields under limited light conditions (100 ft-candles at the surface supplied by fluorescent lights) which appear to be in the same range of magnitude as those found for *Chlorella, Scendesmus,* and *Euglena.*

Under conditions of *Oscillatoria* growth with aerobic bacteria, 95 to 97% of the 5-day BOD is removed. Reduction in COD of 80 to 90% were obtained.

The harvested algae contained 40·9 to 44·4% protein, 13·3 to 25·3% ether-soluble fat, and 6·0 to 6·2% ash when grown in milk waste media.

Based on the results of this study *Oscillatoria* appears to have characteristics which make it potentially satisfactory for waste water stabilization ponds.

BIBLIOGRAPHY

1. Caldwell, D. H., *Sewage Works J. 18,* 433-458 (1946).
2. Myers, J., *Public Works.* 79, 25-27 (1948).
3. Gotaas, H. B. and Oswald, W. J., *Sci Monthly.* 79, 368-378 (1954).
4. Ludwig, H. F. et al., First Progress Report. Univ. Calif. Inst. Engg. Res. Bull. No. 1, Ser. No. 44 (1950).
5. Gotaas, H. B. et. al , Second Progress Report. Univ. Calif. Inst. Engg. Res. Bull. No. 3, Ser. No. 44 (1951).
6. Oswald, W. J. et al., *Sewage and Industrial Wastes.* 25, 692-705 (1953).
7. Gotaas, H. B. and Oswald, W. J., Third Rept. Univ, Calif. Inst. Engg. Res. Bull. No. 4, Ser. No. 44 (1952).
8. Oswald, W. J., et al., *Sewage and Ind. Wastes.* 25, 26-37 (1953).
9. Gotaas, H. B. et al., Fifth Rept. Univ. Calif. Inst. Engg. Res. Bull. No. 5, Ser. No. 44 (1954).
10. Oswald, W. J. and Gotaas, H. B., *Proc. Am. Civil Engrs.* 81, No. 686, 1-27 (1955).
11. Gotaas, H. B. and Oswald, W. J., Utilisation of solar energy for waste utilisation (Presented at the conf. on solar energy The Scientific Basis, Tucson, Ariz) (1955).
12. Gaur, A. C., Pipes, W. O. and Gotaas, H. B., *Jour. Water Pollution Control Fed.* 32, 1060-1065, (1960).

OXIDATION POND EFFLUENTS AND THEIR ROLE IN LAND FERTILITY

By

J. S. S. LAKSHMINARAYANA *and* M. PARABRAHMAM

Central Public Health Engineering Research Institute, Nehru Marg, Nagpur

ABSTARCT

In this paper it has been emphasised that raw sewage containing organic and inorganic plant nutrients applied for growing crops or fishes in ponds should be properly treated before its application.

The method of using algae-bacteria symbiosis as a tool for the purification of sewage has been discussed and described.

In industrial places like Bhilai, Durgapur, Ahemdabad. oxidation ponds for treating part of the city sewage are in existence and Chlorella, Oscillatoria, Scenedesmus, Euglena, Microcystis etc. are the major algal types existing in the oxidation ponds in India.

The use of algae as a manure has also been discussed.

During recent years, increasing attention has been devoted to the application of sewage for agricultural purposes and sewage farming is now practised in most parts of India. It is now definitely established that the use of polluted water for irrigation purposes is injurious to public health. (1) The putrefactive bacteria are widely distributed in polluted waters which contain organic manures of animal origin and the observations on the crops raised on sewage have shown constantly that they all carry superficial infection of pathogens, though they are sterile within. (1) Apart from the above the sewage-grown crops, for example, succulent type of vegetables, may also absorb quite a considerable amount of soluble matter of sewage including the bacterial metabolites, which would be present in solution.

Generally all types of soils including sandy soil continuously irrigated with raw sewage, were found to develop "Sewage Sickness" resulting in a steady decrease in crop yield if proper precautions were not taken. Many irrigated farms if visited and soil samples are collected and examined one can find that the porosity of the soil is considerably reduced and in many cases the incidence of hookworm (Ankylostoma duodenale) is of a very high order as a result of which one has to resort to lime treatment of the soil or some other methods which will help in bringing back the soil to suitable stage for irrigation.

OXIDATION PONDS

The raw sewage contains a lot of nutrients, organic and inorganic, which could be very easily used for irrigation purposes. In might be used for sewage farming for growing crops or fish farming for proteins etc. It is imperative from the above to treat the raw sewage before it is used for irrigation purposes,

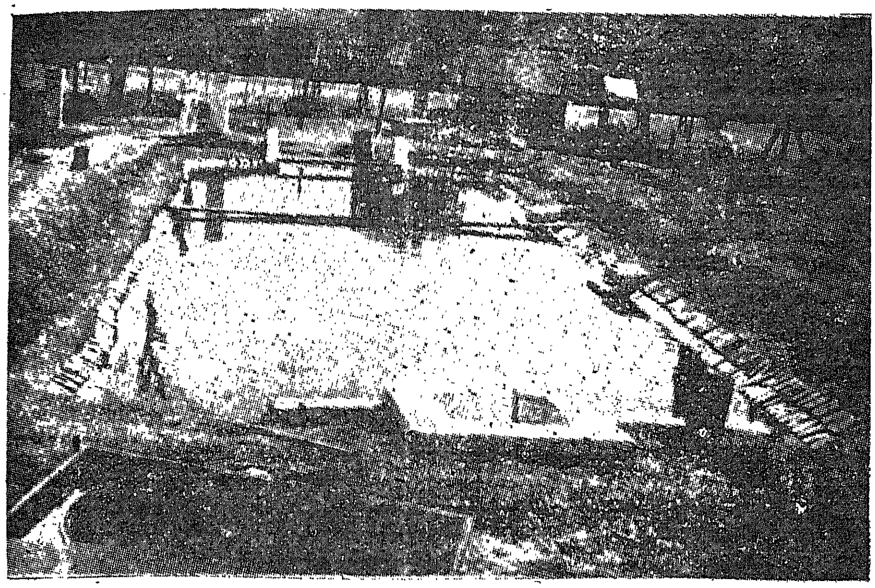
Table I gives the general raw sewage characteristics which are typical to Nagpur, It is easy to observe how the BOD ranges from 120 to 330 parts per

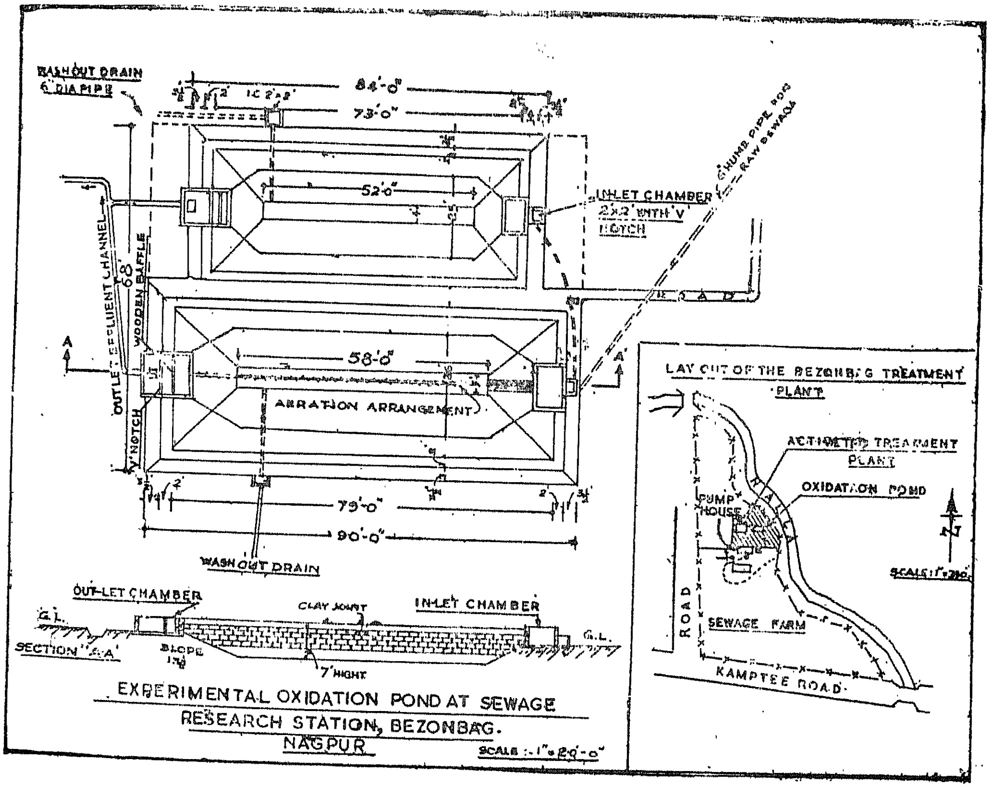
TABLE I

Influent (Raw Sewage) and Effluent Characteristics of Oxidation Ponds, Bezonbagh, Nagpur

	Influent			Effluent			
	Min.	Max.	Av.	Min.	Max.	Av.	
pH	6.9	7·9	7·4	7·7	9·0	8·4	*Pond Volume* 23000 gallons
Total solids (ppm)	400	900	600	280	570	450	Size : *at* 5′ *height* 20′ × 73′
Dissolved solids (ppm)	100	650	450	160	500	310	Slope : 1 : 1:5
Suspended solids (ppm)	50	400	175	25	240	115*	*Detention period* 4 days
Oxygen consumption (ppm) (Tidy figure)	18	60	40	5	28	17	
BOD at 37°C (ppm)	120	330	215	22	90	48	*The effluent sample
				14-F	38-F	22-F	contain algal cells
% Red in BOD	—	—	—	60	90	76	Hence a high figure of value.
Algal cells in millions/ml	—	—	—	0·4	4·8	2·0	
Alkalinity (ppm)	220	284	245	357	215	260	F = Filtered sample
Chloride ,,	50	123	88	65	123	92	
Ca ,,	123	175	143	125	190	153	
Mg ,,	85	114	102	86	140	104	
N (Total) ,,	8	30	16	3	19	9·5	
P ,,	8	14	12	2	5	4	
Na ,,	30	78	53	10	64	38	
K ,,	23	40	33	22	30	29	

million round the year, Till todate, in India, the practice of conventionally treating sewage by activated sludge process or bio-filtration process or primary treatment alone or both by primary and secondary treatment has been very costly and very few cities could afford such a type of costly treatment for their purposes, The method of using algae-bacteria symbiosis as a tool for the purification of sewage is a simple one, commonly known as "oxidation pond treatment", It is very cheap and probably the same results of purification, though not more, when compared to




WASHOUT DRAIN
6" DIA PIPE
84'-0"
73'-0"
52'-0"
IN-LET CHAMBER
2'x2' WITH 'V' NOTCH
OUTLET EFFLUENT CHANNEL
WOODEN BAFFLE
V NOTCH
58'-0"
AERATION ARRANGEMENT
79'-0"
90'-0"
WASHOUT DRAIN
OUT-LET CHAMBER
CLAY JOINT
IN-LET CHAMBER
G.L.
SECTION 'A-A'
7' HIGHT
EXPERIMENTAL OXIDATION POND AT SEWAGE
RESEARCH STATION, BEZONBAG.
NAGPUR
LAY OUT OF THE BEZONBAG TREATMENT PLANT
OXIDATION POND
PUMP HOUSE
ROAD
SEWAGE FARM
KAMPTEE ROAD

conventional treatment plant could easily be achieved. The CPHERI is working on oxidation ponds for the last three and half years and they have come to certain conclusions (2,3) which clearly indicate that the oxidation ponds can form a very cheap treatment for rural, urban and semiurban areas. Further work is in progress to find out the design criteria of these ponds.

The conversion of wastes to algal cell constituents in an oxidation pond involves the symbiotic culture of green algae or mixtures of greens and blue-greens along with bacteria. Bacteria utilise oxygen made available by algae to oxidise and breakdown of organic matter in sewage, During the course of this breakdown, the bacteria release carbon-dioxide and ammonia, and various other products. They, in turn, through energy from sunlight, are converted to new cell material by the photosynthetic activity of the algae. As a result of the combined activities of these microorganisms the organic matter is decomposed, and through photosynthesis ultimately converted to new cellular material, predominantly algal. The process is accomplished by passing sewage through a pond especially constructed to enhance the growth of algal-bacterial population. Figures I and II show the pilot plant oxidation ponds existing at Bezonbagh, Nagpur, and table I indicates the percentage reduction in various physical and chemical factors that are achieved in this pond. In India places like Ahmedabad, Bhilai, Bhopal, Durgapur, Hyderabad, Jodhpur, Bichpuri (Agra) and Madras are having oxidation ponds for treating part of their sewage. Basing on the results obtained at Nagpur and other places, many organisations like the CPWD, NMDC, P. H. E. departments of various states and other private bodies are contemplating to have this type of sewage treatment.

Algal types of Oxidation Ponds Existing in India :

Chlorella, Scenedesmus, Ankistrodesmus, Euglena, Microcystis, Arthospira, Merismopedia, Oscillatoria, are the major algal types of the oxidation ponds existing in India. Forms like Tetraedron, Pandorina, Eudorina, Palmella, Phacus, Chlorogonium, Carteria Chlorococcum, Chlamydomonas and Trachelomonas are some of the minor types of these ponds. All these algae are not nitrogen fixers. The work done at the CPHERI (2) clearly pointed out that the green algae specially Chlorella or mixture of Chlorella, Scenedesmus and Ankistrodesmus donate more oxygen when compared to blue-green types. However, under Indian conditions the blue-green algae may dominate at many of the places. It is necessary to maintain healthy algal blooms in the oxidation ponds. Before starting the ponds one has to prepare unialgal cultures, preferably those of green algae and introduce them in the ponds and gradually build up the algal bloom. The algal bloom could be maintained in the ponds under healthy optimum conditions with proper loadings, maintenance of dissolved oxygen, pH etc. Results obtained so far indicate that the ponds could be worked out successfully at five feet operational depth under Indian conditions and the BOD loadings could be in the range of 500-700 lbs per acre per day.

Nutrient Removals and Algal Yields :

Table I clearly shows the reductions in nitrogen, phosphate, sodium and magnesium from the raw sewage during oxidation pond treatment. The nitrates that have been derived from the raw sewage by the algal activity are stored up in the new algal crop and the analysis of the algae show that proteins range from 35 to 50 percent, carbohydrates 13-25 per cent, fats 13-22 per cent on ash-free dry weight basis. On dry weight basis the CPHERI ponds yield an average of 50 metric tons per acre per year.

Table II shows the composition and yield of Chlorella as a crop in sewage and waste water effluents reported from various places all over the world. Due to the

TABLE II

Composition and Yield of Chlorella as a Crop in Sewage and Waste Water Effluents (Dry Weight Basis)

Chlorella	Japan	California	Cook, 1962 (Scenedesmus & Chlorella)	Germany	Israel	Cpheri
Proteins %	50	41·8	41·8	...	...	35—50
Carbohydrates %	20	21·5	27·4	...	...	13—25
Fats %	...	...	7·2	30—40	...	13—22
Ash %	8—10	19·1	19·1	...	...	8—18
Total yield % (Dry weight) Metric tons/acre/year	13	20—30	...	13	27	50

TABLE III

Rare elements present in Chlorella (Spectrographic Analysis)

Element	Algal ash sample (Dried at 400°C)	Ignited residue (from Bomb Calorimeter)
1. Aluminium	+	+
2. Cobalt	–	Trace
3. Gallium	+	+
4. Germanium	–	Trace
5. Lead	+	+
6. Magnesium	+	+
7. Manganese	+	–
8. Nickel	+	+
9. Phosphorus	+	+
10. Silver	+	–
11. Tin	+	+
12. Zinc	+	+

+ indicate Presence

– indicate Absence

tropical conditions, as evident from the table II the high light intensity that is available in India will make it possible to produce higher quantities of algae from waste water.

The harvested algae possess certain other elements some of which might be useful for enriching the soils. Besides the above nutrients the raw sewage carries lot of amino acids.

Table IV gives the various amino acids that are present in raw sewage as well as in the effluent of activated sludge plant. Lubitz (5) has given the amino acid

TABLE IV

Amino Acids in Raw Sewage and in Activated Sludge
(After Pillai *et. al*, 1953)

Amino acids	Raw Sewage		Activated Sludge	
	In the free form	In the acid hydrolysate	In the free form	In the acid hydrolysate
(1) Cystine	Present	9·6	Nil	19·0
(2) Lysine and histidine	Present	13·3	0·5	48·0
(3) Arginine	Present*	8·7	0·3	26·5
(4) Serine, glycine & aspartic acid	Present*	26·5	0·8	51·5
(5) Glutamic acid & threonine	Present*	34·5	1·0	69·0
(6) Alanine	Absent	14·6	1·1	33·5
(7) Proline	Absent	+	Nil	+
(8) Tyrosine	Present	7·9	0·6	18·7
(9) Tryptophane	Absent	‡	0·7	‡
(10) Methionine and valine	Present*	10·1	1·0	26·5
(11) Phenylalanine	Present	9·4	0·6	17·6
(12) Leucines	Present*	16·4	1·5	38·9

*Present in slightly higher concentrations than in the other cases but not in estimable amounts.

*Present but was not estimated.

†Tryptophane, if present, should have been destroyed during acid hydrolysis.

composition of powdered *Chlorella* (cf. Table V). Cook (6) has given the various vitamins that are present in waste water algal bloom-Scenedesmus and Chlorella pyrenoidosa.

TABLE V

Analysis and Vitamin Content of Dried Powdered Chlorella (after Lubitz, 1963)

Protein (nitrogen × 6·25)	... 55·5 %
Crude fat	... 7·5%
Carbohydrate, total (anthrone method)	... 17·8%
Ash	... 8·25%
Moisture (hot-air oven at 105°C)	... 7·00%
Crude fiber (cellulose and hemicelluose)	... 3·1%
Urea[a]	... 0·08%
Chlorophyll	... 2·68%
Total calories	... 5·2 Kcal/g
Ascorbic acid	... 14·6 mg/100g
Vitamin B_6	... 3·0 μg/g
Thiamine (B_1)	... 7·7 μg/g
Pantothenic acid	... 11·2 μg/g
B-Carotene	... 50·2 mg/100 g

An idea of the various vitamin contents of dried and uncooked green algae can be obtained with certain types of commonly consumed foods (cf. Table VI)

All these indicates that the algae that are obtained from oxidation ponds are the store houses of protein, carbohydrates, fats, amino acids and vitamins which could be easily used either as feed or food provided they are supplied under hygienic conditions. Experiments on chicks and rats carried out by Japanese and US workers have shown that these algae could be used very easily as food. The successful operation of an oxidation pond depends on the production of salable product, i. e., the harvested algae which gives a main economic justification for the use of this method.

[a] Urea was carried over from the growth medium.

Algae as Manure :

The sewage effluents that are coming from the oxidation ponds will be free from most of the pathogenic organisms and coliforms etc. (2,3,7, & 8) and they can be safely used for land irrigation. Pillai and others (9) have shown that the loss of nitrogen from Indian soils that are irrigated by sewage is of very high order. Even nitrogen fixed from the atmosphere is not stable and is steadily lost. The loss of nitrogen from sewage is very rapid. These losses occur under conditions—Aerobic, Semi-aerobic and Annerobic—though the extent of loss is variable and seems to be at a minimum under controlled aerobic conditions. There will be a tendency for the nitrogen level to remain constant after the first fortnight of the application of sewage on the land and under normal conditions the destruction

TABLE VI

Comparison of the Vitamin Content of Dried, Uncooked Green Algae with Certain Types of Commonly Consumed Foods (Amounts are given on the Basis of 100 gm Fresh Weight)

(After Cook, 1962)

Vitamin	Algae	Liver (Beef)	Beef (Round)	Bread (whole wheat)	Spinach (cooked)
INTERNATIONAL UNITS					
Vitamin A	0	43,900	0	0	0
Carotene	1,00,333	0	0	0	9,420
MILLIGRAMS PER CENT					
Ascorbic acid* (total)	39·6	31	0	0	59
Thiamine*	1·15	0·26	0·08	0·30	0·11
Riboflavin*	2·69	3·33	0·17	0·13	0·20
Niacin*	10·8	13·7	4·7	3·0	0·6
Pantothenic acid+(total)	4·60	9·336	0·656	0·789	0·136
Folic acid ‡	72·80	0·294	0·0092	0·029	0·094
Vitamin B_{12} [s]	0·107	116·0	1·93	0	0

Except for the values for nutrients in the algae, the values quoted are from the following sources :

*Watt and Merill 19

+Zook *et. al.*[9]

‡Toepfer, *et. al*[20]

[s]Lichtenstein, *ei. al.*[21]

or the loss of nitrogen increases. Pillai and others (9) found that the major part of the nitrogen is lost in the form of ammonia which occurs most rapidly when septicized sewage is spread over the soil on a warm day. If this loss would be prevented and the nitrogen made fully available to the immediate crop, there would be a greater return in the form of land food.

Oxidation pond effluents are rich in nitrogen contents even though about 50 per cent of nitrogen is taken away by the algae. The algae serve as store houses, as pointed out earlier, for various types of proteins, amino acids etc., and these could be made available to the land gradually if these algae are present in the effluents of the oxidation ponds. After the land is water-logged for some time and irrigated, it increases the porosity of the soil and the algae that are present in the effluents will gradually disintegrate, and the proteins will be made available gradually by the bacteria that are present in the form of nitrogen to the soil. In this way the algae serve as economic organic manures to the soil. By oxidation pond treatment the effluents are made quite safe from the public health point of view. If the farmer is educated about the safety of the oxidation pond effluents he would eventually use the same for the irrigation of his lands.

Besides the availability of algal nutrients to the soil, the atmospheric carbon dioxide fixation in the form of carbohydrates and proteins in the algae will form an additional source of carbon for the land. The use of algae (non-nitrogen fixers) as organic manures for land fertility has to be experimented further to substantiate the above observations. To reach this goal further work is in progress.

ACKNOWLEDGEMENTS

The authors acknowledge with thanks the keen interest taken by Shri R. S. Mehta, Director, C. P. H. E. R. I., and for his kind permission to present this paper.

REFERENCES

1. Pillai, S. C., Rajagopalan, R. and Subrahmanyam, V. 1955. Sewage Farming and Sewage-grown Crops in relation to Health. *Bull. National Inst. Sci. of India*, (10) 160-179.

2. CPHERI, Nagpur, 1961. Studies on Oxidation Ponds Carried out at Sewage Research Station of CPHERI at Bezonbagh, Nagpur. *CPHERI Bulletin* 3 (3), 119-134.

3. Lakshminaryana, J. S. S., Parabrahman, M., Khan, A. N., and Ratnaparkhi, D. Y. 1963 Domestic Sewage Oxidation Ponds at Nagpur (India). *Proc. of Waste Treatment by Oxidaation Ponds,* held at CPHERI during October 29-30.

4. Pillai, S. C., Mohanrao, G. J., Krishnamurty, K., and Prabhakara Rao, A. V. S. 1953. Amino Acids in Sewage and Activated Sludge. *Curr. Sci.* 22, 235.

5. Lubitz, A. J. 1963. The Protein Quality, Digestibility and Composition of Alage, Chlorella 71105. *Jour. Food. Sci.* 28, (2), 229-232.

6. Cook, B. B. 1962. The Nutritive Value of Waste-grown Algae. *Amer. Jour. Public Health,* 52 (2), 243-251.

7. Cooper, R. C. 1962. Some Public Health Aspects of Algal-bacterial Nutrient Recovery Systems. 52, (2), 252-257.

8. Parhad, N. M. and Rao, N. U. 1963. Preliminary Studies on the Microbiology of Oxidation Ponds Proc. of Waste Treatment by Oxidation Ponds held at CPHERI during October 29-30.

9. Pillai, S. C. Rajagopalan, R. and Subrahmanyam, V. 1946. Loss of Nitrogen from Sewage. *Curr. Sci.*, 15, 290-91.

NITROGEN ENRICHMENT OF RICE SOILS BY BLUE GREEN ALGAE AND ITS EFFECT ON THE YIELD OF PADDY

By

R. SUBRAHMANYAN, L. L. RELWANI *and* G. B. MANNA

Central Rice Research Institute, Cuttack

ABSTRACT

Experimental results show that blue green algae could be utilised in enriching water-logged rice fields for producing paddy.

Preliminary results show that application of algae is better than a dose of 60 kgms. of amonium sulphate per hectare.

INTRODUCTION

The ability of several bacteria and blue green algae to fix atmospheric nitrogen is now fairly well known. Considerable fundamental work has been done on these since two decades, in Japan, U. S. A., India and U. S. S. R. Most of the nitrogen fixing bacteria are, however, not active in the water-logged condition of growth of the rice plant; the blue green algae which are ubiquitious in their distribution, therefore, assume an importance under these conditions.

The large number of investigations carried out with blue green algae in pot cultures of rice plant has proven the beneficial role of these algae in supplying the nitrogen requirements of the rice plant. But, systematic investigations on a field scale employing these algae are not many as to reassure the cultivator for adoption of the method.

The problems connected with raising large quantities of required species of algae are stupendous, same as those connected with harvesting the enormous quantities of plankton produceed in an aquatic environment. The money to be expended will be uneconomical.

Therefore, at the Central Rice Research Institute, we have laid emphasis on practical field experiments and the approach has been:—

1. To strengthen indigenous blue green algal flora of the paddy soil with application of fertilizer mixture of lime + super phosphate + trace of sodium molybdate;
2. After burning of the top soil ("rabbing") to eliminate as much of the indigenous flora as possible to introduce known nitrogen fixing forms and preparing the soil to receive the same; and
3. Employment of single or mixtures of species grown in the laboratory under conditions as close to nat ure as possible.

Nitrogen in any form was not applied in any of the treatments except in a separate treatment for comparison in terms of the popular fertilizer, ammonium sulphate.

By employing the above procedure, it is found that only a few grams of alage per hectare are required. The algae are uniformly distributed mixing them with sand for the purpose, after transplanting the seedlings. The details of the lay-ont of field experiments may be had from earlier publication (Relwani 1963, and Relwani and Subrahmanyan. 1963).

RESULTS

The results obtanied during three successive seasons at C. R. R. I. are tabulated together with the treatments and yields (Table I(*a*) and I(*b*). The popular

TABLE 1-(*a*)

Year-wise grain yields of paddy (Three crops—1962 Main season, 1962-63 Second season and 1963 Main season)

Serial no.	Treatments	Pot culture experiments (gm. per pot) Average of five replications					Field experiments (Kg. per hectare) Average of four replications.				
		1962 Main season	1962-63 Second season	1963 Main season	Mean	Percentage on control	1962 Main season	1962-63 Second season	1963 Main. season	Mean	Percentage on control
1	A—Control (No. manure)	10·43	6·30	13·26	10·00	100·0	2379·47	459·78	1769·81	1536	100·0
2	B—Rabbing (Partial soil sterilization)	29·34	17·18	25·25	23·92	239·2	2857·82	818·20	2496·17	2057	133·9
3	C—A + Algae	10·81	7·58	13·38	10·59	105·9	2578·39	658·03	2194·66	1810	117·8
4	D—B + Algae	27·04	16·06	32·66	25·25	252·5	2977·87	879·24	2620·22	2159	145·6
5	E—Fertilizer mixture*	11·03	9·15	20·48	13·55	135·5	2942·09	789·61	3000·88	2244	146·1
6	F—E + Rabbing	33·31	19·14	36·34	29·60	296·0	3489·41	1033·25	3170·07	2564	166·9
7	G—E + Algae	15·67	21·11	50·63	29·14	291·4	3441·15	978·92	3979·12	2799	182·2
8	H—E + Algae + Rabbing	39·17	20·61	55·31	38·36	383·6	3661·25	1070·77	3833·81	2855	185·8
9	I—Ammonium sulphate	11·30	10·88	27·61	16·60	166·0	3002·59	819·32	2463·08	2095	136·3
	C. D. (0·05) to compare treatment means :	4·30	3·59	4·65	...	...	139·60	153·31	352·31	...	...
	C. D. (0·10) to compare treatment means :	5·77	4·83	6·25	...	...	189·27	207·77	477·54	...	...

*Lime @ 1000 Kg., Super phosphate @ 20 Kg. P_2O_5 and sodium molybdate @ 0·28 Kg. per hectare.

TABLE I-(*b*)

Average grain yields of paddy (Average of three crops—1962 main crop 1962-63 second crop and 1963 main crop)

Fertilizer and soil sterilization treatments	Pot culture experiments				Field experiments			
	No. algae	Percentage on control	Algae	Percentage on control	No. algae	Percentage on control	Algae	Percentage on control
Control (No-manure)	10·00	100·0	10·59	105·9	1536	100·0	1810	117·8
Partial soil sterilization or rabbing	23·92	239·2	25·25	252·5	2057	133·9	2159	145·6
Fertilizer mixture	13·55	135·5	29·14	291·4	2244	146·1	2799	182·2
Rabbing and fertilizer mixture	29·60	296·0	38·36	383·6	2564	166·9	2855	185·8
Ammonium Sulphate	16·60	166·0	...	...	2095	139·3	...	...

varieties of paddy T. 141 (145 days duration) and Ptb. 10 (110 days duration) were used as test crops for the main (July-December) and second (January-April) crops respectively.

As may be seen from Tables I(*a*) and (*b*), both under pot culture and field conditions. the average response to blue green algal inoculation over a basal dressing of fertilizer mixture consisting of lime + super + sodium molybdate was maxium as evidensed by the extra grain yields of 155·9% in pots and 36·1% in the field, which is equivalent to 20 Kg N/Ha applied as ammonium sulphate. (It may be mentioned here that such a difference between the pot and field results is obviously due to the fact that environment conditions are not identical).

The increased yields when fertilizer mixture is applied is due to the activation of the indigenous flora. The effect of *rabbing* (partial soil sterilization) was found to be considerable both in the pots and field. However, algae alone have contributed an extra grain yield of 17.8% under field conditions though under pot culture it is only 5·9% It is quite clear from the yield data recorded yearwise, that the trend of response due to various treatments was almost alike under pot and field conditions and different crop seasons.

On the basis of the above findings, another field experiment has been initiated since 1963 (main season) in which the efficiency of blue green algae is being tested alone and in combination with different popular organic manures and fertilizers like farm yard manure, green manure, urea and ammonium sulphate, over a no manure control, all treatments being super-imposed over a basal dressing of lime at 500 kg, super phosphate at 20 kg P_2O_5 and sodium molybdate at 0.28 kg/ha. Grain yields are presented in Table II.

TABLE II

Yield of paddy grains (1963 main season) in Kg/Ha.
Variety T.141 (145 days)

Manures and Fertilizers	No-Algae	Percentage on no-manure control	Algae	Percentage on no-manure control
No-manure control	2615	100	3403	130 **
Farm Yard Manure	3392	130	3573	137
Green Manure (Dhaincha)	3907	149	3902	149
Urea	3366	129	3585	137*
Ammonuim Sulphate	3431	131	3472	132

C. D. (0·05) to compare treatment means = 215 Kg/Ha.

C. D. (0·10) to compare treatment means = 291 Kg/Ha.

* Significant statistically at 5 per cent level.

** Significant statistically at 1 per cent level.

The data show that inoculation of blue green algae alone increases the grain yield significantly by about 30% over the corresponding control treatment and it is found to be statistically on the same level as 20 kg of nitrogen applied as F. Y. M., urea and ammonium sulphate. None of the manures or fertilizers has been found to enhance the activity of blue green algae except urea.

CONCLUSIONS

The data presented above appear to be very encouraging and we feel that blue green algae could be employed to enrich N-fertility of water-logged rice field soils leading to beneficial results. Some preliminary experiments have shown that apart from enriching the soil with nitrogen, they also release some growth promoting substances, for, a little application of algae gives a better yield than nitrogen as ammonium sulphate at even 60 kg/ha level.

ACKNOWLEDGEMENT

We thank Dr. R. H. Richharia, Director, C. R. R. I. for all interest and encouragement in these investigations.

REFERENCES

Relwani, L. L., Role of blue green alage on paddy yield. *Current Science*, 32:417—18 (1963).

Relwani, L. L. and R. Subrahmanyan. Role of blue green algae, chemical nutrients and partial soil sterilization on paddy yield. *Ibid.*, 32:441—43 (1963)